

**THE EFFECTS OF DEGRADING PERINEURONAL NETS IN THE MEDIAL
PREFRONTAL AND POSTERIOR PARIETAL CORTICES ON THE SPATIAL
WORKING MEMORY OF RATS**

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ABSTRACT

Perineuronal nets (PNNs) are specialized extracellular matrix structures that surround subsets of neurons in the central nervous system (CNS). They help in maintaining a stable excitatory-inhibitory balance in the brain, and the adult loss of PNNs can lead to a period of increased synaptic plasticity. Furthermore, the loss of PNNs can affect cortical networks and influence learning, memory, and cognition. The aim of this thesis was to test the effect that degrading PNNs in the medial prefrontal (mPFC) and posterior parietal (PPC) cortices had on spatial working memory (WM). To do this, the spatial WM of Long-Evans rats was measured using the trial unique, delayed nonmatching-to-location (TUNL) task in touchscreen-equipped operant conditioning chambers. Rats were trained in this task and then assigned to either a penicillinase (PEN) control or chondroitinase ABC (ChABC) treatment. ChABC is an enzyme that compromises the structure of PNNs by degrading one of their major components: chondroitin sulfate proteoglycans (CSPGs). Surgeries were performed to infuse these enzymes into the medial prefrontal cortex (mPFC) in a first set of rats and into the posterior parietal cortex (PPC) in a second set of rats. All rats were trained under a standard 6 s delay and then tested under 4 conditions: a 6 s delay, a variable 2 s or 6 s delay, a 2 s delay with a 1s inter-trial interval (interference condition), and a 20 s delay. Rats that received mPFC ChABC infusions initially performed better than controls in the 20 s delay condition, but did not perform any differently in any of the other three conditions. Rats that received PPC ChABC infusions did not perform significantly differently from controls in any condition. Immunohistochemical analysis confirmed that CSPGs were degraded in both cortical regions. This suggests that PNNs in the mPFC are involved in learning a novel delay in a spatial WM task, but that they are not essential for general spatial WM function. Furthermore, it appears that PNNs in the PPC are not involved in spatial WM. Ultimately, these findings contribute to a growing body of literature that explores how cortical PNNs are involved in cognition.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AP	Anteroposterior
CANTAB	Cambridge Neuropsychological Test Automated Battery
ChABC	Chondroitinase ABC
CNS	Central Nervous System
CSPGs	Chondroitin Sulfate Proteoglycans
C4S	Mouse Anti-Chondrotin-4-Sulfate
DNMP	Delayed Nonmatching-to-Position
DV	Dorsoventral
ECM	Extracellular Matrix
GABA	γ -Aminobutyric Acid ^[1-1] _{SEP}
GABAergic	Pertaining to or Affecting γ -Aminobutyric Acid
HA	Hyaluronan
ITI	Inter-trial Interval
MMP	Matrix Metalloprotease
mPFC	Medial Prefrontal Cortex
PEN	Penicillinase
PFC	Prefrontal Cortex
PNN	Perineuronal Net
PPC	Posterior Parietal Cortex
PV	Parvalbumin
PV+	Parvalbumin Producing
TUNL	Trial Unique, Nonmatching-To-Location
V1	Primary Visual Cortex
WFA	<i>Wisteria Floribunda</i> Agglutinin
WM	Working Memory

1.0 INTRODUCTION

1.1 *Spatial Cognition and Spatial Working Memory*

Spatial cognition refers to the acquisition, organization, revision, and application of knowledge regarding one's spatial surroundings (Burgess 2008). Central to spatial cognition is spatial working memory (WM), which is the ability to temporarily store and reorganize visual information (Awh and Jonides 2001). Two processing systems are thought to underlie spatial WM: allocentric and egocentric processing (Burgess 2006). These processing systems are crucial for an organism to gather visual information from their environment and effectively navigate in their surroundings. Allocentric navigation relies on outside cues, and tracks the location of objects with respect to other objects (Burgess 2006). Egocentric navigation relies on internal cues, and tracks objects relative to one's own body (Burgess 2006). In humans, imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography have been paired with computer-based tasks to test what brain regions are involved in spatial WM (Jonides et al. 1993; D'Esposito et al. 1998). This imaging technology has helped us to better understand the cortical network that underlies spatial WM. Additionally, new imaging technology has important implications with regards to studying CNS disorders, since patients with autism, schizophrenia, and epilepsy commonly have deficits in spatial WM (Rahko et al. 2016; Starc et al. 2017; Reyes et al. 2018). In rats, several tasks have been developed that test spatial WM, including the Morris Water maze, T maze, Y maze, radial arm maze, and delayed non-matching to sample tasks (Dudchenko 2004). From research in humans, primates, and rats, it has been made clear that the prefrontal cortex (PFC), hippocampus, and posterior parietal cortex (PPC) play important roles in spatial WM (Eriksson et al. 2015). Further research is required to understand exactly how neurons communicate between the PFC, hippocampus, and PPC, as well as other brain areas, in maintaining functional spatial cognition.

1.2 *Medial Prefrontal Cortex*

In humans, the frontal lobe is at the front of the brain and is classified as one of the brain's four cortical lobes, with the others being the parietal, temporal, and occipital lobes. The prefrontal cortex (PFC) is found in the most anterior part of the frontal lobe and can be subdivided into caudal, lateral (dorsolateral and ventrolateral), medial, and orbitofrontal regions

(Murray et al. 2016). As its name suggests, the medial prefrontal cortex (mPFC) exists medially within the PFC.

Functionally, the rat PFC appears to combine features of the human orbitofrontal and dorsolateral prefrontal cortices (Uylings et al. 2003). It is interconnected with many other brain regions, including cortical, subcortical, and brain stem sites (Alvarez and Emory 2006). For example, bidirectional projections between the mPFC and amygdala modulate anxiety-related and social behaviors (Felix-Ortiz et al. 2016). Additionally, many types of memory rely on communication between the mPFC and the hippocampus (Wirt & Hyman 2017), and executive control relies on fronto-parietal circuits (Eriksson et al. 2015). Furthermore, the mPFC is also involved in decision-making (Walton et al. 2002), impulsivity (Narayanan et al. 2006), attention (Kim et al. 2016b), the maintenance of emotional responses (Smith et al. 2018), and learning (Rushworth et al. 2011). For the purposes of this thesis, however, the remainder of this section will focus on the role that the mPFC plays in learning and memory.

One way that the mPFC contributes to learning and memory is through the regulation of motivation. Motivation largely influences attention and the capacity for an organism to learn (Lang 1995). Studies in humans and rats have shown that the ventral mPFC is tied to the motivation related to the anticipation of an outcome (Rushworth et al. 2011). This is consistent with the fact that the ventral mPFC communicates with the hypothalamus, which has been strongly implicated with emotion and motivation (Reppucci and Petrovich 2016). In rats, the mPFC mediates motivation in the context of a food reward (Petrovich et al. 2007), but also other forms of motivation such as voluntary wheel running (Basso and Morrell 2015). The mPFC is also involved in memory consolidation. Inactivation of the mPFC either several weeks before or soon after the introduction of a memory task leads to memory impairment (Tuscher et al. 2018; Leon et al. 2010). If the mPFC is inactivated long after the introduction of a memory task, memory is not impaired (Carballo-Màrquez et al. 2007). Therefore, the role of the mPFC in memory consolidation is timing-specific.

The mPFC is also involved in spatial WM. The role of the mPFC in spatial WM appears to be quite specific, as was found by a group that tested the spatial WM of mPFC-lesioned rats using visuospatial conditional discrimination in a Y maze (DelaTour and Gisquet-Verrier 1999). They found that when trained on a 5 s delay, mPFC lesions impaired the performance of rats when they were switched to a novel 20 s delay. Interestingly, mPFC-lesioned rats that were

trained using a variable 0, 5, 10, or 20 s delay were not impaired when switched to a non-variable 20 s delay (Gisquet-Verrier et al. 2000). This implies that the mPFC is involved in adapting to a novel WM delays. To conclude, the mPFC is an integral brain region in humans, monkeys, and rodents. Notably, it plays a key role in many forms of WM, including spatial WM.

1.3 Posterior Parietal Cortex

The posterior parietal cortex (PPC) is part of the parietal lobe, and is located posterior to the primary somatosensory cortex. It forms connections to areas throughout the brain, including the medial prefrontal cortex, orbitofrontal cortex, somatosensory cortex, striatum, thalamus, and visual areas (Whitlock 2017). The PPC is involved in visual decision-making, planned movement, spatial attention, spatial navigation, and spatial WM (Licata et al. 2017; Whitlock 2017). This has been demonstrated repeatedly in primate research, with fewer rodent studies tying the PPC to these cognitive functions. Damage to the PPC, particularly in the right hemisphere, can result in hemispatial neglect, which refers to the inability to orient or respond to a meaningful stimulus presented to one side of the body or visual field (King and Corwin 1993). This phenomenon has been observed in rodents, monkeys, and humans (King and Corwin 1993). Notably, abnormalities in PPC function have also been tied to the cognitive deficits in certain CNS disorders. For example, neuroimaging studies found that patients with Alzheimer's disease showed hypometabolism and hypoperfusion in the PPC (Foster et al. 1984; Jacobs et al. 2012). Additionally, an fMRI study recently found that dysfunction of the PPC in human schizophrenia patients is associated with decreased WM storage among these patients (Hahn et al. 2018). To conclude, the PPC is an important brain region that plays an integral role in spatial cognition. Additionally, the PPC appears to be involved in CNS disorders, and there is a growing pool of evidence that suggests that the PPC of rats is analogous in function to the PPC of primates. This makes the PPC of rats an intriguing target when studying the cognitive circuits underlying spatial working memory.

1.4 Comparing the Medial Prefrontal Cortex and the Posterior Parietal Cortex

The mPFC and PPC are connected by the superior longitudinal fasciculus and the dorsal cingulum bundle, which are tracts that interconnect the frontal, parietal, and temporal lobes in the human brain. WM is thought to rely on circuits that connect the frontal and parietal cortices,

and therefore one would expect the mPFC and the PPC to contribute to spatial WM in similar ways. However, with multiple pathways connecting the mPFC and PPC, it would be an oversimplification to assume that the two brain regions share identical functions with regards to WM. For example, the mPFC is involved in many forms of WM, whereas the PPC seems to primarily be involved in spatial and visual WM (Compton et al. 1994; Berryhill and Olson 2008; Licata et al. 2017; Scott et al. 2019). Additionally, one group tested rats with either mPFC or PPC lesions on five different spatial WM tasks: a Mumby delayed nonmatching-to-position (DNMP), Aggleton DNMP, Morris Water maze, Landmark Water maze, and radial arm maze (Kolb et al. 1994). Interestingly, rats with mPFC lesions were impaired on all five tasks, whereas rats with PPC lesions were only impaired in the Morris and Landmark Water mazes. Therefore, the mPFC and the PPC appear to have unique but important contributions to spatial WM.

1.5 Trial Unique, Nonmatching-To-Location Task

In this thesis, rats were trained and tested in the automated operant-conditioned touchscreen TUNL task. The Trial Unique, Nonmatching-To-Location (TUNL) task is based on a DNMP paradigm used to test the spatial WM of rodents (Rivalan et al. 2017). It does, however, differ from the standard DNMP task in several ways. Typically, a DNMP task begins with a sample phase where rats are presented with a single lever in one of two possible positions. They must press against the lever, and once successful a delay period ensues. This is followed by a choice phase where a single lever is presented at each of the two locations. To correctly perform the task and receive a reward, rats must press the novel lever that was presented, otherwise known as the non-matched lever. This tests spatial WM because it requires the rat to encode and apply visual information regarding the location of the presented lever over a short delay (Dudchenko et al. 2013). A limitation of this task is that rats can develop mediating strategies, which can inflate their performance and reduce their reliance on spatial WM during the task (Panlilio et al. 2011). For example, rats can orient their bodies towards the to-be-correct lever during the delay period. Alternatively, rats can focus their attention to one lever but press down the opposite lever, which does not accurately reflect their choice in the task.

The automated operant-conditioned touchscreen TUNL task was developed in order to address these kinds of limitations (Rivalan et al. 2017). Like the DNMP paradigm, in TUNL stimuli are presented at two locations in any given trial. Unlike it, however, stimuli appear in 14

possible areas in a 7x2 grid of squares rather than 2 in DNMP. In order to interact with the 7x2 grid, rats must press down a response shelf with their paws. Because of this, rats are inclined to nose poke and therefore draw their gaze to the illuminated stimulus. Furthermore, each stimulus appears randomly in 1 of the 14 possible squares, which makes it impossible to predict the correct location of the non-matched stimulus prior to the choice phase (Talpos et al. 2010). The length of the delay and the distance between sample and choice stimuli can also be manipulated in the TUNL task. Longer delay periods challenge WM more than shorter delay periods, and small distances between stimuli challenge pattern separation more than large distances between stimuli. Pattern separation in the context of TUNL refers to the ability to keep two similar but distinct patterns as separate representations within the mind (Talpos et al. 2010). Finally, the automated nature of the TUNL task reduces researcher bias during testing, is more time-efficient, and reduces variability amongst trials.

There is evidence that the TUNL task involves brain regions such as the mPFC, the hippocampus, and the PPC (Talpos et al. 2010; McAllister et al. 2013; Josey and Brigman 2015; Kim et al. 2015; Davies et al. 2017; Scott et al. 2019). This is consistent with the findings that all three of these brain areas are involved in spatial WM. Lesions to the mPFC lead to a delay-dependent impairment in spatial WM, without an impairment in spatial pattern separation in the TUNL task (McAllister et al. 2013). This has been demonstrated by testing rats with either a minimal or a 6 s delay period, whereby mPFC-lesioned rats were only impaired in the 6 s delay condition. Furthermore, mPFC-lesioned rats were not impaired in small or large separations when a minimal delay was present. This suggested no effect on spatial pattern separation. In the same study, mPFC-lesioned rats also had increased susceptibility to interference as shown by decreased performance in a condition that utilized a minimal delay and inter-trial interval (ITI) compared to sham controls (McAllister et al. 2013). Unlike mPFC lesions, hippocampal lesions lead to both an impairment in delay-dependent WM and in pattern separation (Talpos et al. 2010). PPC inactivation also impairs the performance of rats in the TUNL task (Scott et al. 2019). Therefore, like many other spatial WM tasks, TUNL relies on the hippocampus, mPFC, and PPC. Unlike many other tasks that involve a nonmatching paradigm, however, TUNL allows for the careful manipulation of stimulus separations and automated randomization of delays, which can elucidate more specific roles of cortical regions in spatial WM.

1.6 *Perineuronal Nets*

Perineuronal nets (PNNs) are specialized extracellular matrix structures that surround specific subsets of neurons throughout the central nervous system. Hyaluronan (HA) makes up the backbone of PNNs, and is covalently linked to chondroitin sulfate proteoglycans (CSPGs). This structure is stabilized by tenascin-R and various PNN link proteins, many of which are also found in cartilage (Deepa et al. 2006). These molecules combine to form a rigid, lattice-like structure that surrounds neurons, limiting structural changes and their ability to form new neuronal connections (Kwok et al. 2010). PNNs exist throughout the CNS in relatively low numbers early in development, and grow throughout postnatal life (Rogers et al. 2018). This growth highly coincides with the closure of critical periods, whereby synaptic plasticity is reduced and stabilization of a long-term synaptic network occurs (Pizzorusso et al. 2002). Additionally, PNNs support neurons in ion buffering, cell signaling, and in the protection against oxidative stress (Morawski et al. 2004; Brückner et al. 1993; Dzyubenko et al. 2016). Because of their supportive role, the degradation of PNNs may lead to neuronal dysfunction and cognitive abnormalities. This can impede learning and memory formation (Banerjee et al. 2017), or disrupt intact memories (Hylín et al. 2013; Xue et al. 2014). Conversely, PNN degradation can also lead to memory enhancements (Romberg et al. 2013). This dichotomy illustrates the complex relationship that PNNs have with cognition, and highlights their importance in functional cognition.

Multiple CNS disorders present with abnormal PNN expression in the brain. For example, post-mortem analysis studies suggest that cortical PNN levels are reduced in schizophrenia and Alzheimer's disease (Baig et al. 2005; Franklin et al. 2008; Mauney et al. 2013; Enwright et al. 2016). Consistent with this, reduced cortical PNN levels have been observed in animal models of CNS disorders (Pollock et al. 2014; Paylor et al. 2016; Wen et al. 2018). This is interesting, because PNNs are heterogeneous structures whose distribution varies based on the brain region, age, experience, and species under investigation (Shen 2018; Lensjø et al. 2017). PNNs exist across many vertebrate species, which implies that they play an integral role in the CNS. Also, PNN reductions have been observed in both animal models of CNS disorders and patients with CNS disorders, which make PNNs an intriguing therapeutic target.

Over the years, many ways of targeting PNNs have been developed. Chondroitinase ABC (ChABC) and hyaluronidase are two enzyme treatments that acutely degrade PNNs by targeting

two of their major components, CSPGs and HA, respectively (Frischknecht et al. 2009). Interestingly, PNNs have the ability to regrow over time after ChABC and hyaluronidase infusions. One study tracked PNN regrowth in the primary visual cortex (V1) of Long-Evans rats using a *Wisteria floribunda* agglutinin (WFA) stain, and found that it took approximately 60 days for the PNNs to fully regrow following ChABC infusions into the V1 (Lensjø et al. 2017). A significant advantage of using ChABC and hyaluronidase as treatments is that they can acutely degrade PNNs in a specific brain region. Unfortunately, however, these treatments do not specifically degrade PNNs. For example, only 2% of CSPGs (the main target of ChABC) in the CNS are concentrated in PNNs (Fawcett, 2015). Additionally, CSPGs have inhibitory activity outside of PNNs, which means that behavioural outcomes after ChABC treatment could be the result of a combination of CSPG and PNN degradation (Dou and Levine 1994; Jones et al. 2003). Another way of manipulating PNNs is by targeting matrix metalloproteinases (MMPs), which can be endogenously expressed by PNN-ensheathed neurons (Rossier et al. 2015). MMPs such as MMP-9 degrade perineuronal ECM, and their levels can be modulated to either increase or reduce PNN levels (Gray et al. 2008). Like ChABC and hyaluronidase, MMPs target multiple structures in the ECM and do not specifically degrade PNNs (Bonnans et al. 2014). Some groups have developed knockouts of PNN components to introduce a more specific way of targeting PNNs. By knocking out PNN link proteins such as *crtl1* in rodents, PNN development can be attenuated without altering CSPGs (Carulli et al. 2010). Therefore, knockout models introduce a specific way to target PNNs and can mimic long-term developmental deficits of PNNs as seen in human CNS disorders. Unfortunately, however, one notable disadvantage of these knockout models is that they do not specifically target a single brain region, and instead lead to whole-brain PNN attenuation. To conclude, many different methods have been developed that alter PNN levels, each with their own advantages and disadvantages. Each method targets PNNs in a different way, and has the potential to isolate specific contributions that PNNs and other ECM structures have in memory and cognition. In the present study, ChABC was chosen because we wanted to explore the effect that acutely degrading PNNs specifically in the mPFC and PPC had on cognition. Furthermore, it made sense to use ChABC for the sake of consistency, because the present study follows up on a previous experiment in which ChABC was used to degrade PNNs in the mPFC (Paylor et al. 2018).

PNN degradation in the brain has shown promise in treating some of the cognitive

deficits seen in rodent models of CNS dysfunction. For example, ChABC was used in two different rodent models of Alzheimer's disease to restore memory. One group treated the perirhinal cortex of a P301S transgenic Alzheimer's model of mice, which restored object recognition memory (Yang et al. 2015). Similarly, another group treated the hippocampus of an Alzheimer's mouse model with mutations in its *APP* gene with ChABC, leading to a restoration of contextual memory (Végh et al. 2014). PNNs also appear to play a role in epilepsy (Rankin-Gee et al. 2015; Pollock et al. 2014). Interestingly, ChABC treatment in a rodent epilepsy model increases the number of seizures that occur (Rankin-Gee et al. 2015), while MMP inhibition diminishes PNN degradation, leading to a reduction in seizures (Pollock et al. 2014). PNN degradation has also been performed in fear conditioning and drug-addiction paradigms, yielding unique results. ChABC infusions in the amygdala leads to the erasure of remote fear memories (Gogolla et al. 2009) and drug-related memories (Xue et al. 2014).

ChABC has also been very promising in treating motor impairments caused by stroke and spinal cord injuries. Damage to the spinal cord can lead to glial scarring, in which inhibitory CSPGs are up-regulated and neural regeneration is inhibited (Spijker and Kowk 2017). ChABC infusions into the spinal cord not only degrade PNNs in the area, but also degrade glial scarring following spinal cord injury, which can promote the sprouting of intact and injured spinal systems and the functional recovery of limb movements (Bradbury et al. 2002; Grist et al. 2006). Additionally, ChABC can restore diaphragm function and normal breathing following cervical hemisection of rats (Warren et al. 2018). Sensimotor and limb function recovery following stroke was also improved by ChABC infusions into the spinal cord (Soleman et al. 2012; Wiersma et al. 2017). In conclusion, PNNs are an important part of the ECM and contribute to normal physiological function. Interestingly, their levels are reduced in some CNS disorders. Studying the effect of brain-region specific PNN degradation can help us to understand the role of PNNs in cognitive circuits, and reveal a potential role of PNNs in cognitive dysfunction.

1.7 *Parvalbumin Expressing Neurons*

Parvalbumin (PV) is an EF-hand calcium-binding protein that is found in fast-firing nerve cells in the brain and fast-contracting muscles (Cates et al. 1999). PV-producing (PV+) neurons are one of the major subtypes of interneurons that exist in the cortex, and are found throughout the brain. The majority of PV+ neurons are GABAergic interneurons and are involved in the fast

and powerful inhibition of excitatory pyramidal cells (Celio 1986). Additionally, PV+ neurons in the cortex contribute to the synchronization of neuronal activity, particularly in the gamma frequency range (30-100Hz) (Uhlhaas et al. 2009). This synchronous oscillatory activity of neurons firing together plays an important role in learning and memory formation (Jutras and Buffalo 2010). For example, PV+ interneurons in the PFC play a crucial role in WM, attention, and cognitive flexibility (Murray et al. 2015; Kim et al. 2016a; Kim et al. 2016b). Furthermore, multiple human CNS disorders such as Alzheimer's, autism, and schizophrenia show reductions in PV+ interneurons in the prefrontal cortex (Arai et al. 1987; Beasley & Reynolds 1997; Hashemi et al. 2017). Consistent with this, animal models of CNS disorders have reduced PV+ neuronal density, along with diminished oscillatory and functional activity among prefrontal PV+ neurons (Lodge et al. 2009; Chen et al. 2018). It has been proposed that oxidative stress on PV+ interneurons in the cortex can underlie the PV+ reduction and pathophysiology of some CNS disorders (Powell et al. 2012; Sun et al. 2016; Khan et al. 2017). Interestingly, a lack of PV expression levels among PV+ neurons, rather than a reduction in PV+ neuronal density, has been linked with reduced social interactions and communication in mice (Wöhr et al. 2015). This suggests that it is not only PV+ neuronal density, but also functional PV expression among PV+ neurons that can influence cognition.

Different species appear to have relatively different counts of PV+ neurons in their brains. For example, when comparing GABA+ interneuron co-localization with calcium binding proteins, GABA+ interneurons in the mPFC of rats associate most with PV, whereas GABA+ interneurons in the mPFC of monkeys and humans associate most with calretinin (CR) (Gabbot et al. 1997a; Gabbot et al. 1997b; Gabbot & Bacon 1997c). Both rodents and primates have high levels of co-localization between PV+ neurons and PNNs in the cortex, and the maturation of PNNs highly coincides with that of PV+ interneurons (Härtig et al. 1992; Caballero et al. 2014). Like PNNs, PV+ neurons are highly regulated throughout development and are modulated in an experience-dependent manner (Hensch 2005). For example, physical exercise during adolescence leads to increased hippocampal PV expression in rodents (Gomes de Silva et al. 2010). Additionally, a recent study found that Brevican, a core protein in PNNs, controls the synaptic and cellular plasticity of PV+ neurons by regulating the localization of receptors in their extracellular membranes (Favuzzi et al. 2017). Furthermore, PNNs support the function of PV+ neurons and affect their PV expression (Yamada et al. 2015; Morawaski et al. 2004; Cabungcal

et al. 2013). Conversely, PV⁺ interneurons also appear to affect PNN expression, since PV⁺ interneurons that are enwrapped in PNNs express metalloproteases that can regulate PNN density (Rossier et al. 2015). To conclude, it appears that PNN dysfunction can lead to dysfunction of the GABAergic PV⁺ cells that they ensheath, and that cortical PV⁺ neurons and PNNs appear to have a reciprocal and tight relationship to one another.

1.8 Rationale and Hypotheses

In the current study, the spatial WM of Long-Evans rats was tested following the degradation of PNNs in the mPFC and PPC. Spatial WM was measured using the automated touchscreen TUNL task, which was chosen because it is clinically translatable, requires little handling of animals during testing, and gives an accurate measure of task parameters (Talpos et al. 2010). Additionally, correct performance of the TUNL task relies on the mPFC and the PPC (McAllister et al. 2013; Scott et al. 2019). Likewise, in humans it has been suggested that the frontal cortex and PPC are essential for functional spatial WM, and that WM relies on intact fronto-parietal circuits (Courtney et al. 1998; Alekseichuk et al. 2016; Todd and Marois 2004; Mackey et al. 2016; Eriksson et al. 2015). The TUNL task is an effective way to measure the contribution of PNNs in the mPFC and PPC on the spatial WM of rats, which can give a better idea of how PNNs may influence human cognition. In previous studies, the knockout of PNN components (Montag-Sollaz and Montag 2003), as well as ChABC infusions into the mPFC (Paylor et al. 2018) has led to cognitive impairments. Based on this literature, we hypothesized that the degradation of PNNs in the mPFC and PPC would disrupt the neurological circuits that are required for spatial WM and impair the performance of rats in the TUNL task. More specifically, based on previous observations following mPFC inactivation, we predicted that ChABC infusions into the mPFC would impair TUNL performance in a delay-dependent manner, have no effect on pattern separation, and increase the susceptibility of rats to proactive interference (Delaunay & Gisquet-Verrier 1999; McAllister et al. 2013; Hvoslef-Eide et al. 2015). Because spatial WM has been proposed to depend on fronto-parietal circuits (Eriksson et al. 2015), we predicted that ChABC infusions into the PPC would also impair the performance of rats in TUNL.

2.0 MANUSCRIPT

2.1 Title Page

ChABC infusions into medial prefrontal cortex, but not posterior parietal cortex, improve the performance of rats tested on a novel, challenging delay in the touchscreen TUNL task

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* The following sections (2.2 *Abstract* to 2.6 *Materials and Methods*) are the text of a manuscript submitted to Learning & Memory. The manuscript contains all the experiments described and completed for this master's thesis. *

2.2 Abstract

Perineuronal nets (PNNs) are specialized extracellular matrix structures that surround subsets of neurons throughout the central nervous system (CNS). They are made up of chondroitin sulfate proteoglycans (CSPGs), hyaluronan, tenascin-R, and many other link proteins that together make up their rigid and lattice-like structure. Modulation of PNNs can alter synaptic plasticity and thereby affect learning, memory, and cognition. In the present study, we degraded PNNs in the medial prefrontal (mPFC) and posterior parietal (PPC) cortices of Long-Evans rats using the enzyme chondroitinase ABC (ChABC), which cleaves apart CSPGs. We then measured the consequences of PNN degradation on spatial working memory (WM) with a trial-unique, non-matching-to location (TUNL) automated touchscreen task. All rats were trained with a standard 6 s delay and 20 s inter-trial interval (ITI) and then tested under four different conditions: a 6 s delay, a variable 2 s or 6 s delay, a 2 s delay with a 1 s ITI (interference condition), and a 20 s delay. Rats that received mPFC ChABC treatment initially performed TUNL with higher accuracy, more selection trials completed, and fewer correction trials completed compared to controls in the 20 s delay condition, but did not perform differently from controls in any other condition. Rats that received PPC ChABC treatment did not perform significantly differently from controls in any condition. Posthumous immunohistochemistry confirmed an increase in CSPG degradation products (C4S stain) in the mPFC and PPC following ChABC infusions to those respective brain regions. These findings suggest that PNNs in the mPFC play a subtle role in spatial WM, but PNNs in the PPC do not. Furthermore, it appears that PNNs in the mPFC are involved in adapting to a challenging novel delay, but that they do not play an essential role in the standard TUNL task with a 6 s delay.

2.3 Introduction

PNNs are part of the extracellular matrix (ECM) of the central nervous system (CNS). They are composed of chondroitin sulfate proteoglycans (CSPGs), hyaluronan, tenascin-R, and many link proteins, which make up their rigid matrix structure (Deepa et al. 2006). PNNs act as a physical barrier to structural changes in the neurons that they surround (Kwok et al. 2010). Additionally, PNNs are involved in synaptic stabilization and in the closure of critical periods of plasticity during development (Pizzorusso et al. 2002). The density of PNNs is relatively low early in development and increases throughout postnatal life (Rogers et al. 2018). Related to this, the removal of PNNs in adulthood can reopen periods of heightened plasticity comparable to that of a juvenile critical period (Lensjø et al. 2016). With such heightened plasticity comes potential reorganization of neuronal structure, connectivity, and function (Pizzorusso et al. 2002).

PNNs appear to be involved with learning and memory in the adult brain, and PNN degradation in the brain can lead to changes in behaviour and cognition (Gogolla et al. 2009; Lee et al. 2012; Xue et al. 2014; Happel et al. 2014; Thompson et al. 2018; Lasek et al. 2018; Paylor et al. 2018). A common method of degrading PNNs is by targeting CSPGs with the enzyme chondroitinase ABC (ChABC) (Lee et al. 2009). Interestingly, the cognitive and behavioural changes following ChABC infusions depend on the location and timing of treatment (Shen 2018). For example, the infusion of ChABC into the perirhinal cortex enhances recognition memory in an object recognition paradigm (Romberg et al. 2013). Conversely, the infusion of ChABC into the medial prefrontal cortex (mPFC) leads to impaired cross-modal object recognition and object oddity tests (Paylor et al. 2018). Furthermore, PNN distribution in the CNS varies between species, and therefore PNN disruptions may cause varied behavioural effects across different animal models (Lensjø et al. 2017). However, the conservation of PNNs across many vertebrate species implies that they play an integral and similar function in the CNS. Additionally, reductions in cortical PNNs have been observed in patients with schizophrenia and Alzheimer's disease (Baig et al. 2005; Mauney et al. 2013; Enwright et al. 2016) and in animal models of stroke, prion diseases, epilepsy, and schizophrenia (Hobohm et al. 2005; Franklin et al. 2008; McRae et al. 2012; Paylor et al. 2016). Continued investigation of how PNNs contribute to cognition in adults can help us to discover whether there is a link between abnormalities in PNN distribution and the cognitive deficits seen in these CNS disorders.

In the current study, the spatial working memory (WM) of Long-Evans rats was tested

following the degradation of PNNs in the mPFC and posterior parietal cortex (PPC). Spatial WM was measured using the automated touchscreen trial-unique, non-matching-to location (TUNL) task, which was chosen because it is clinically translatable, requires little handling of animals during testing, and gives an accurate measure of task parameters (Talpos et al. 2010). Performance of the TUNL task relies on the mPFC and the PPC (McAllister et al. 2013; Scott et al. 2019). Likewise, in humans it has been suggested that the frontal cortex and PPC are essential for functional spatial WM, and that WM relies on intact fronto-parietal circuits (Courtney et al. 1998; Alekseichuk et al. 2016; Todd and Marois 2004; Mackey et al. 2016; Eriksson et al. 2015). In our previous study, ChABC infusions into the mPFC led to cognitive impairments (Paylor et al. 2018). Based on these findings, we hypothesized that the degradation of PNNs in the mPFC and PPC would disrupt the neurological circuits that are required for spatial WM and impair the performance of rats in the TUNL task.

2.4 Results

Rats were trained in the TUNL task (Figures 1 & 2) until they reached a minimum group average of 50 trials at 65% accuracy using a 6 s delay. Surgeries were then performed, infusing penicillinase (PEN) control or ChABC into the mPFC of one cohort of rats and into the PPC of another cohort of rats. After 5-10 days of recovery, rats were tested in TUNL under 4 different conditions: a 6 s delay, a variable 2 s or 6 s delay, a 2 s delay with a 1 s ITI (interference condition), and a 20 s delay (Figure 1).

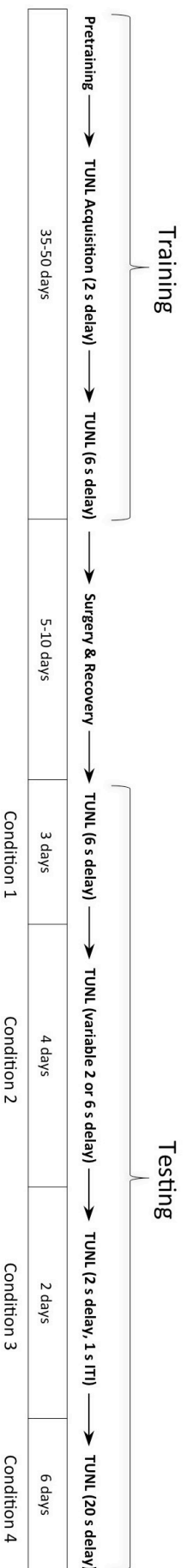


Figure 1.1: Schematic of the training and testing schedule. During the training phase, rats were trained on TUNL to criterion. Chondroitinase ABC or penicillinase was then locally infused into medial prefrontal cortex or posterior parietal cortex. Following recovery, rats were re-tested for 3 days on the standard TUNL task that they were trained on pre-surgery (Condition 1). During Condition 2, a variable delay 2- or 6-second delay was used to assess working memory. Condition 3 was designed as an interference task with a 1-second, instead of the standard 20-second, ITI between trials. Condition 4 introduced a novel challenging delay of 20 s.

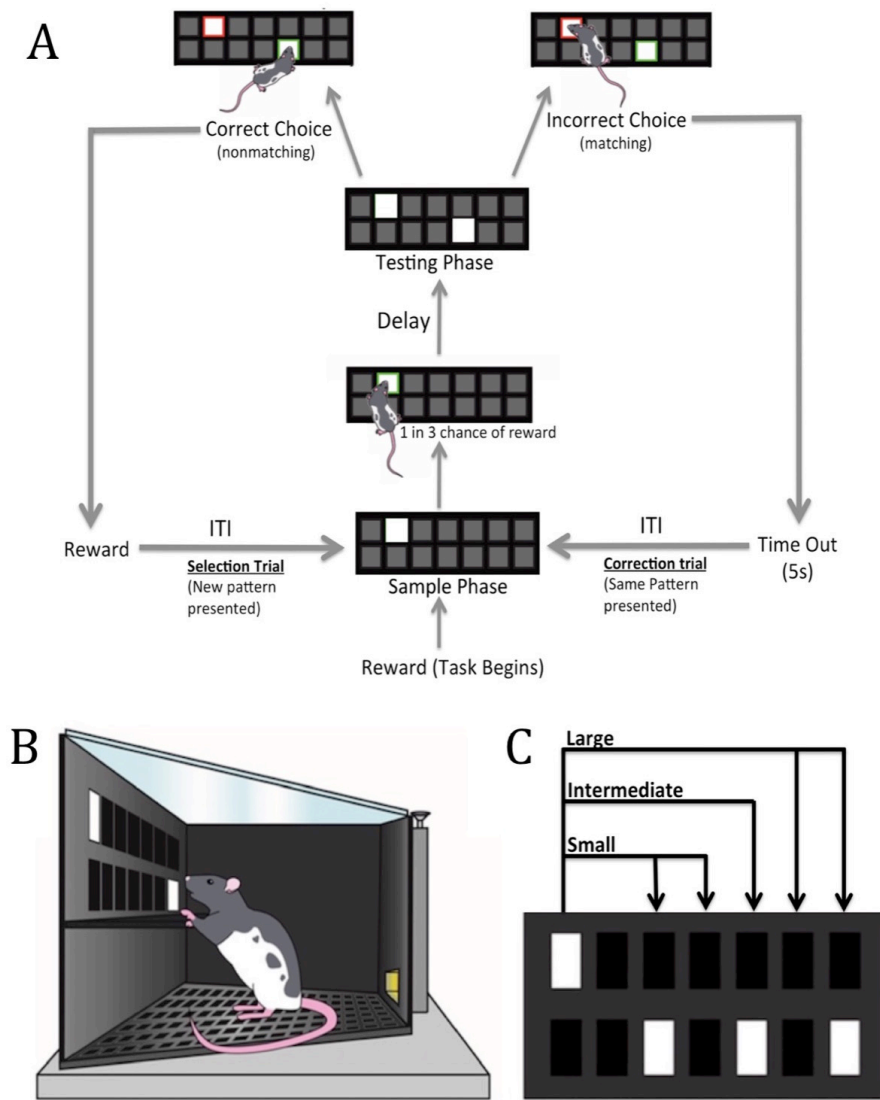


Figure 1.2: (A) Schematic showing the progression of a TUNL trial. The standard TUNL task that rats are trained on uses a 6 s delay and a 20 s ITI, but the length of both of these periods can be altered. (B) Schematic of a Long Evans rat performing the testing phase of the TUNL task. (C) Schematic labeling distances between stimuli in the testing phase as large (4-5 squares away), intermediate (3 squares away), or small (1-2 squares away). Stimuli within the testing phase of a trial are not presented horizontally, vertically, or diagonally adjacent to one another.

2.4.1 ChABC infusions into the mPFC improve performance of the TUNL task with a 20 s delay

ChABC infusions in the mPFC had no effect on the performance of rats in condition 1 (6 s delay)

A mixed 2x2 ANOVA (treatment, separation) revealed that the accuracy of PEN and ChABC-treated rats was not significantly different (Fig. 3A; $F(1,21) = 0.00$, $p = 0.99$). The percent accuracy of rats was higher on trials with large stimulus separations compared to trials with small stimulus separations ($F(1,21) = 101.31$, $p < 0.001$), and there was no interaction between stimulus separation and treatment ($F(1,21) = 0.12$, $p = 0.73$). Independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 3B; $t(21) = 0.60$, $p = 0.56$), selection trials completed (Fig. 3C; $t(21) = 0.67$, $p = 0.51$), correction trials completed (Fig. 3D; $t(21) = -0.117$, $p = 0.91$), or reward latency (Fig. 3E; $t(21) = 0.51$, $p = 0.62$). A mixed 2x2 ANOVA (treatment, latency type) revealed that there was no difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 3F; $F(1,21) = 0.11$, $p = 0.74$), but that the incorrect trial latency was significantly longer than the correct trial latency ($F(1,21) = 15.86$, $p = 0.001$). There was no significant interaction between treatment and latency type ($F(1,21) = 1.61$, $p = 0.22$).

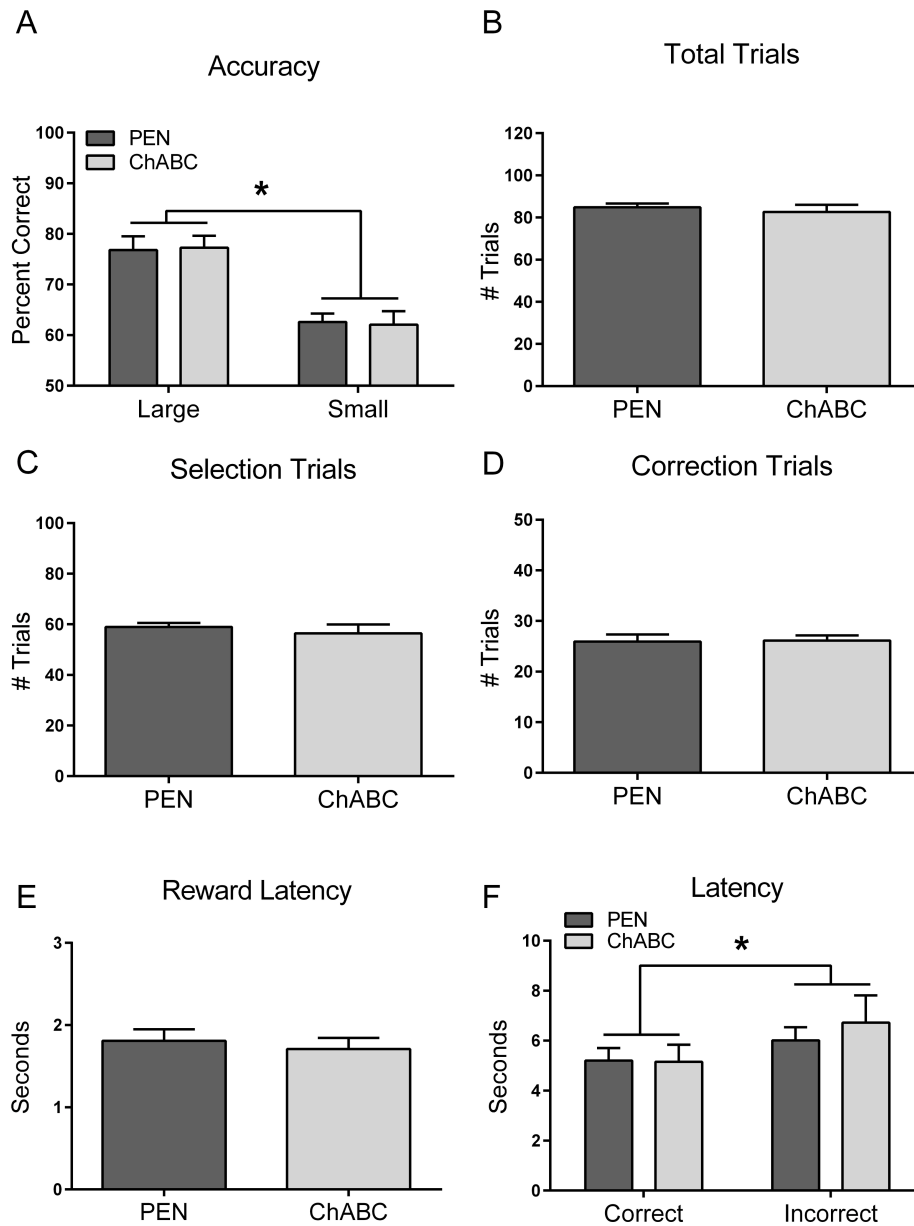


Figure 1.3: Effects of PEN or ChABC infusions into the mPFC on TUNL with a 6 s delay (Condition 1). All values represent the average (Mean \pm SEM) of the testing days (A) ChABC had no effect on accuracy. Accuracy was significantly higher on trials with large stimulus separations compared to trials with small stimulus separations. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on the latency of correct and incorrect trials. Incorrect latency was significantly longer than correct latency. PEN, $n = 12$; ChABC, $n = 11$; $*p < 0.05$.

ChABC infusions in the mPFC had no effect on the performance of rats in condition 2 (variable 2 s or 6 s delay)

A mixed three-way ANOVA (treatment, separation, delay) revealed no significant differences in percent accuracy between ChABC and PEN-treated rats (Fig. 4A; $F(1,21) = 0.07$, $p = 0.80$). However, main effects of separation ($F(1,21) = 8.64$, $p = 0.008$) and delay ($F(1,21) = 131.32$, $p < 0.001$) were found, as well as a significant interaction between the two factors ($F(1,21) = 87.12$, $p < 0.001$). Post hoc analyses using Tukey's method revealed that the percent accuracy on trials with a 2 s delay and small stimulus separations was significantly greater than any other combination of delay and separation ($p < 0.05$). Additionally, the percent accuracy on trials with a 2 s delay and small stimulus separations was not significantly different than that of trials with a 6 s delay and small stimulus separation ($p > 0.05$), but both were significantly different from that of trials with a 6 s delay and large stimulus separation ($p < 0.05$) (Fig. 4A). There were no significant interactions between treatment and delay ($F(1,21) = 0.16$, $p = 0.69$), treatment and separation distance ($F(1,21) = 0.00$, $p = 0.95$), or treatment x delay x separation distance ($F(1,21) = 0.39$, $p = 0.54$).

Independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 4B; $t(21) = 1.18$, $p = 0.25$), selection trials completed (Fig. 4C; $t(21) = 1.43$, $p = 0.17$), correction trials completed (Fig. 4D; $t(21) = 0.22$, $p = 0.83$), or reward latency (Fig. 4E; $t(21) = -0.272$, $p = 0.79$). A mixed 2x2 ANOVA (treatment, separation) revealed no significant difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 4F; $F(1,21) = 0.81$, $p = 0.38$), but the incorrect trial latency was significantly longer than the correct trial latency ($F(1,21) = 16.17$, $p = 0.001$). There was no significant interaction between treatment and latency type ($F(1,21) = 1.38$, $p = 0.25$).

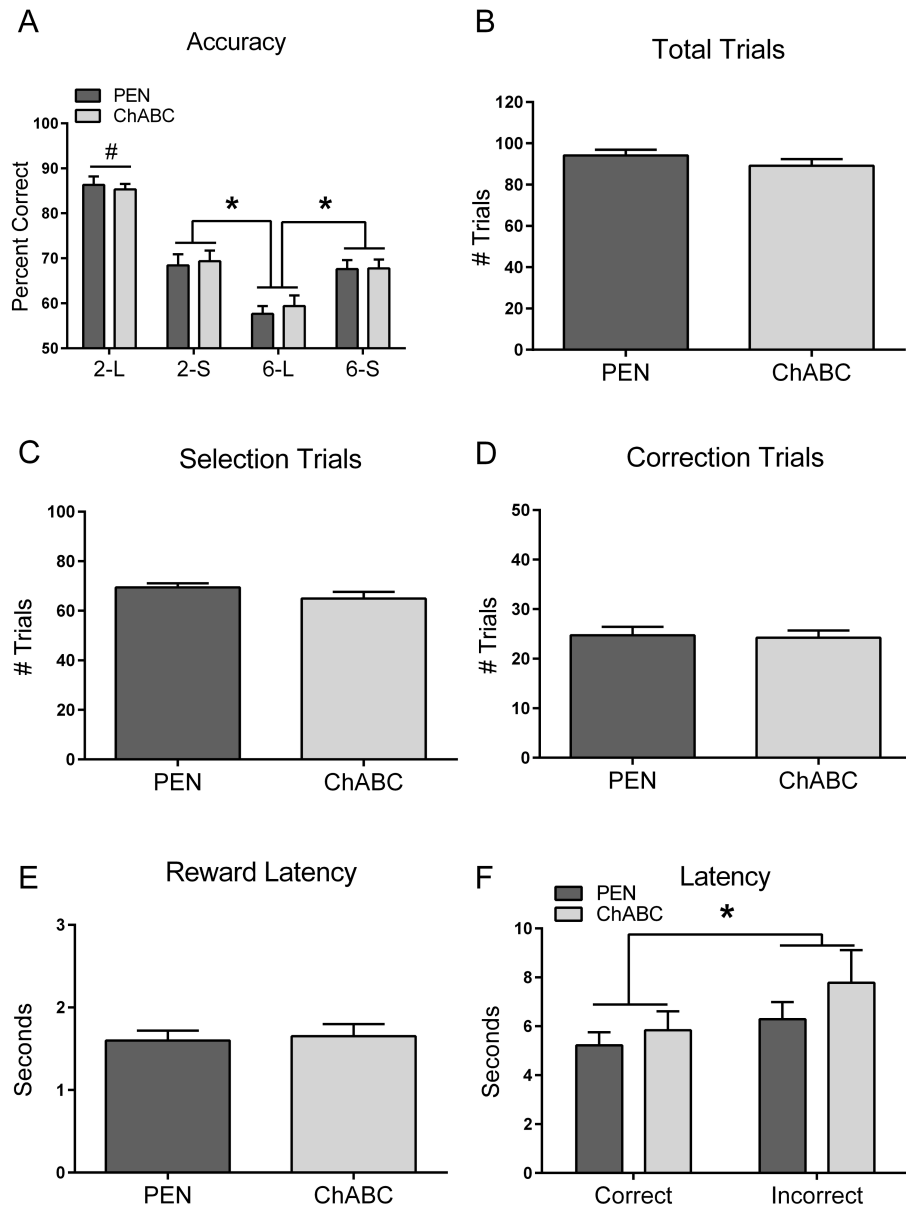


Figure 1.4: Effects of PEN or ChABC infusions into the mPFC on TUNL with a variable 2 s or 6 s delay (Condition 2). All values represent the average (Mean \pm SEM) of the testing days. (A) Percent accuracy on trials with either a 2 s delay and large stimulus separation, 2 s delay and small stimulus separation, 6s delay and large stimulus separation, and 6 s delay and small stimulus separation. ChABC had no effect on percent accuracy. Percent accuracy on trials with a 2 s delay and a large stimulus separation was significantly higher than any other group. Percent accuracies on trials with a 2 s and 6 s delay with small stimulus separations were not significantly different from one another, but were both significantly higher than percent accuracy on trials with a 6 s delay and a large stimulus separation. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on correct or incorrect latency. Incorrect latency was significantly longer than correct latency. PEN, $n = 12$; ChABC, $n = 11$; # significantly different from all other groups ($p < 0.05$); * $p < 0.05$.

ChABC infusions in the mPFC had no effect on the performance of rats in condition 3 (2 s delay with a 1 s inter-trial interval)

A mixed 2x2 ANOVA (treatment, separation) revealed that the percent accuracy of ChABC-treated rats was not significantly different than that of PEN-treated rats (Fig. 5A; $F(1,21) = 0.00$, $p = 0.94$). However, the percent accuracy of rats was significantly higher on large separation trials compared to small separation trials ($F(1,21) = 189.54$, $p < 0.001$), with a significant interaction existing between stimulus separation and treatment ($F(1,21) = 5.46$, $p = 0.029$). Independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 5B; $t(21) = -0.41$, $p = 0.68$), selection trials completed (Fig. 5C; $t(21) = -0.33$, $p = 0.75$), correction trials completed (Fig. 5D; $t(21) = -0.27$, $p = 0.79$), or reward latency (Fig. 5E; $t(21) = -0.14$, $p = 0.89$). A mixed 2x2 ANOVA (treatment, latency type) revealed no significant difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 5F; $F(1,21) = 0.45$, $p = 0.51$), but that the incorrect trial latency was significantly longer than the correct trial latency ($F(1,21) = 13.62$, $p = 0.001$). There was no significant interaction between treatment and latency type ($F(1,21) = 1.49$, $p = 0.24$).

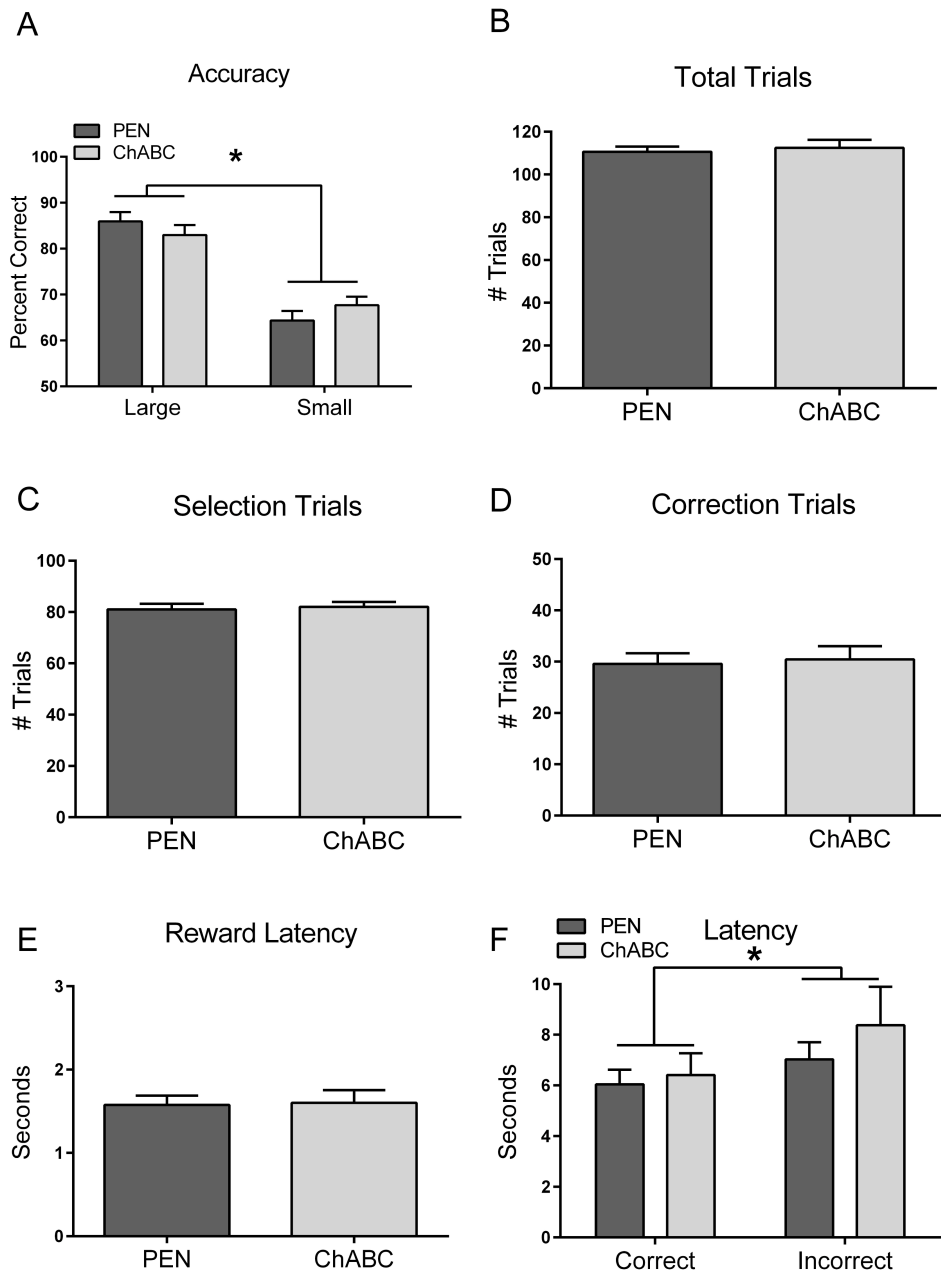


Figure 1.5: Effects of PEN or ChABC infusions into the mPFC on TUNL with a 2 s delay and a 1 s ITI (Condition 3). All values represent the average (Mean \pm SEM) of the testing days. (A) ChABC had no effect on percent accuracy. Percent accuracy on trials with large stimulus separations was significantly higher than percent accuracy on trials with small stimulus separations. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on correct or incorrect latency. Incorrect latency was significantly longer than correct latency. PEN, $n = 12$; ChABC, $n = 11$; * $p < 0.05$.

ChABC infusions in the mPFC improved performance of rats in condition 4 on the first two testing days (20 s delay)

Overall accuracy, accuracy at large stimulus separations, accuracy at small stimulus separations, total trials completed, selection trials completed, and correction trials completed were analyzed with mixed 2x3 ANOVAs (treatment, testing day). Overall percent accuracy was not significantly different between PEN and ChABC-treated rats (Fig. 6A; $F(1,21) = 3.00$, $p = 0.10$). Additionally, there was no main effect of testing day ($F(2,42) = 2.66$, $p = 0.08$), and no interaction between treatment and testing day ($F(2,42) = 1.19$, $p = 0.32$). The percent accuracy for trials with large stimulus separations was not significantly different between PEN and ChABC-treated rats (Fig. 6B; $F(1,21) = 1.60$, $p = 0.22$). Furthermore, there was no main effect of testing day ($F(2,42) = 1.60$, $p = 0.21$) and no interaction between treatment and testing day ($F(2,42) = 2.30$, $p = 0.11$). The percent accuracy for trials with small stimulus separations was not significantly different between PEN and ChABC-treated rats (Fig. 6C; $F(1,21) = 2.14$, $p = 0.16$). There was also no main effect of testing day ($F(2,42) = 1.13$, $p = 0.33$), and no interaction between treatment and testing day ($F(2,42) = 0.42$, $p = 0.66$).

There was no significant difference in the number of total trials completed by PEN and ChABC-treated rats (Fig. 6D; $F(1,21) = 2.93$, $p = 0.10$). Additionally, there was no main effect of testing day ($F(2,42) = 0.69$, $p = 0.51$), and no interaction between treatment and testing day ($F(2,42) = 2.15$, $p = 0.13$). There was also no significant difference in the number of selection trials completed by PEN and ChABC-treated rats (Fig. 6E; $F(1,21) = 0.42$, $p = 0.53$). However, there was a significant main effect of testing day ($F(2,42) = 9.95$, $p < 0.001$), and a significant interaction between treatment and testing day ($F(2,42) = 4.37$, $p = 0.019$). Post hoc analyses using Tukey's method revealed that rats performed significantly more mean selection trials on testing days 12-13 compared to testing days 10-11 ($p < 0.05$), as well as significantly more mean selection trials on testing days 14-15 compared to testing day 10-11 ($p < 0.05$).

There was no difference in the number of correction trials completed by PEN and ChABC-treated rats (Fig. 6F; $F(1,21) = 3.60$, $p = 0.07$). There was, however, a significant main effect of testing day ($F(2,42) = 15.53$, $p < 0.001$), but no interaction between treatment and testing day ($F(2,42) = 2.69$, $p = 0.079$). Post hoc analyses using Tukey's method revealed that rats performed fewer correction trials on testing days 12-13 ($p < 0.05$) and 14-15 ($p < 0.05$) compared to testing days 10-11.

An independent-samples t-test revealed that PEN and ChABC-treated rats did not have any significant differences in reward latency (Fig. 6G; $t(21) = -0.30$, $p = 0.77$). A mixed 2x2 ANOVA (treatment, latency type) revealed no significant difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 6H; $F(1,21) = 0.82$, $p = 0.38$), but the incorrect trial latency was significantly longer than the correct trial latency ($F(1,21) = 9.34$, $p = 0.006$). There was no interaction between treatment and latency type ($F(1,21) = 2.59$, $p = 0.12$).

When investigating the initial two days of the 20 s delay condition (testing days 10-11), independent samples t-tests revealed that ChABC-treated rats initially had a higher total percent accuracy (Fig. 6A; $t(21) = -3.38$, $p = 0.003$), higher large separation percent accuracy (Fig. 6B; $t(21) = -2.74$, $p = 0.012$), more selection trials completed (Fig. 6E; $t(21) = -2.31$, $p = 0.031$), and fewer correction trials completed (Fig. 6F; $t(21) = 3.52$, $p = 0.002$) compared to PEN-treated rats. There were no significant differences in small separation percent accuracy (Fig. 6C; $t(21) = -1.528$, $p = 0.14$), total trials completed (Fig. 6D; $t(21) = 0.92$, $p = 0.37$) between ChABC-treated rats and PEN-treated rats over the first 2 days of the 20 s delay.

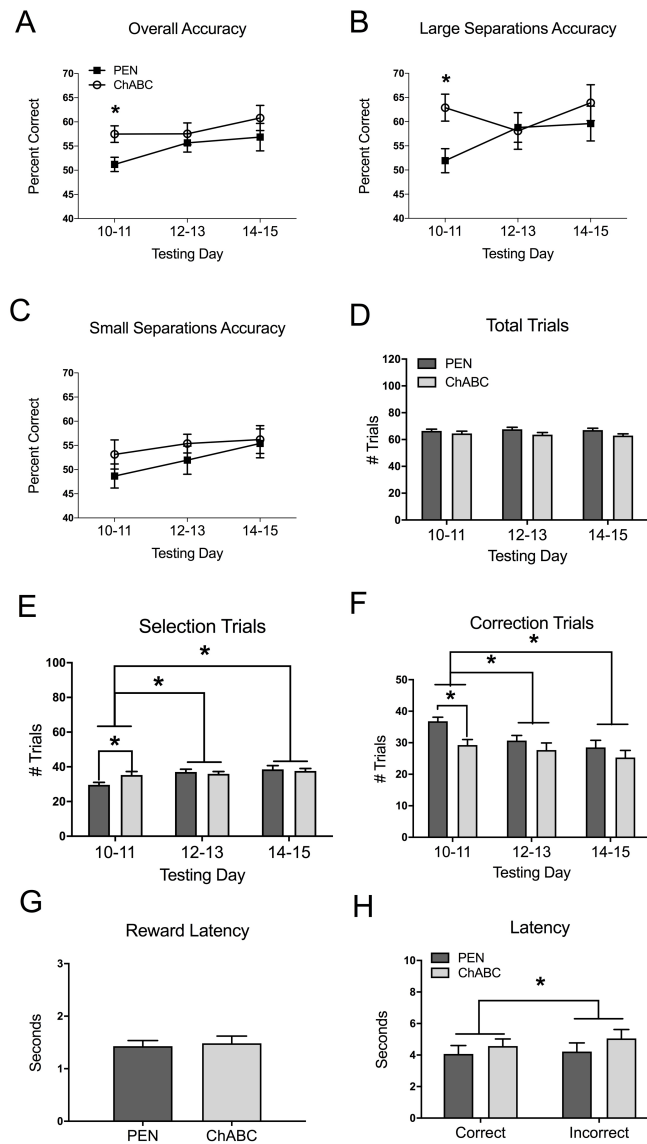


Figure 1.6: Effects of PEN or ChABC infusions into the mPFC on TUNL with a 20 s delay period (Condition 4). All values represent the average (Mean \pm SEM) of the testing days. (A) ChABC-treated rats had a significantly higher overall percent accuracy than PEN-treated rats over the first two days of testing, but not over the full six days of testing. (B) ChABC-treated rats had a significantly higher percent accuracy on trials with large stimulus separations compared to PEN-treated rats over the first two days of testing, but not over the full six days of testing. (C) ChABC had no effect on percent accuracy in trials with small stimulus separations. (D) ChABC had no effect on the number of total trials completed. (E) ChABC-treated rats completed significantly more selection trials than PEN-treated rats over the first two days of testing, but not over the full six days of testing. Rats performed significantly more selection trials on testing days 12-13 and 14-15 compared to testing days 10-11. (F) ChABC-treated rats completed significantly fewer correction trials than PEN-treated rats over the first two days of testing, but not over the full six days of testing. Rats performed significantly fewer correction trials on testing days 12-13 and 14-15 compared to testing days 10-11. (G) ChABC had no effect on reward latency. (H) ChABC had no effect on correct or incorrect latency. Incorrect choice latency was significantly higher than correct choice latency. ChABC infusions had no effect on reward latency. PEN, $n = 12$; ChABC, $n = 11$; * $p < 0.05$.

2.4.2 ChABC infusions into the PPC does not alter performance of rats in the TUNL task

ChABC infusions in the PPC had no effect on the performance of rats in condition 1 (6 s delay)

A mixed 2x2 ANOVA (treatment, separation) revealed that the accuracy of PEN and ChABC-treated rats was not significantly different (Fig. 7A; $F(1,22) = 3.48$, $p = 0.08$). Rats performed better on trials with large stimulus separations compared to trials with small stimulus separations ($F(1,22) = 18.34$, $p < 0.001$), and there was no interaction between stimulus separation and treatment ($F(1,22) = 0.25$, $p = 0.62$). Independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 7B; $t(22) = 0.93$, $p = 0.36$), selection trials completed (Fig. 7C; $t(22) = 1.29$, $p = 0.21$), correction trials completed (Fig. 7D; $t(22) = -0.12$, $p = 0.91$), or reward latency (Fig. 7E; $t(22) = -1.76$, $p = 0.09$). Additionally, a mixed 2x2 ANOVA (treatment, latency type) revealed that there was no difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 7F; $F(1,22) = 0.73$, $p = 0.40$), and that there was no difference in correct and incorrect trial latencies ($F(1,22) = 2.50$, $p = 0.13$). There was no significant interaction between treatment and latency type ($F(1,22) = 0.20$, $p = 0.66$).

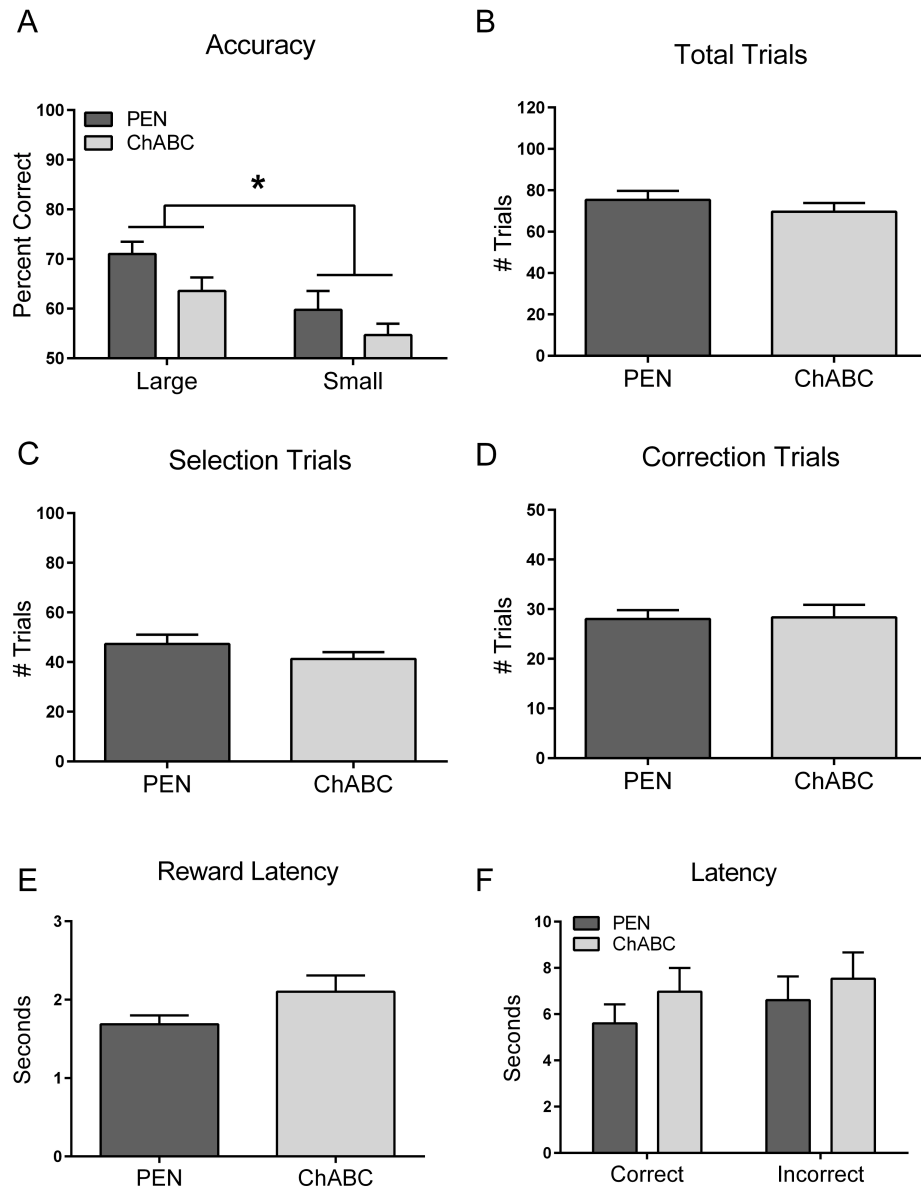


Figure 1.7: Effects of PEN or ChABC infusions into the PPC on TUNL with a 6 s delay (Condition 1). All values represent the average (Mean \pm SEM) of the testing days. (A) ChABC had no effect on accuracy. Accuracy was significantly higher on trials with large stimulus separations compared to trials with small stimulus separations. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on the latency of correct and incorrect trials. There was no difference in correct and incorrect latency. PEN, $n = 12$; ChABC, $n = 12$; * $p < 0.05$.

ChABC infusions in the PPC had no effect on the performance of rats in condition 2 (variable 2 s or 6 s delay)

A mixed three-way ANOVA (treatment, separation, delay) revealed no significant differences between ChABC and PEN-treated rats (Fig. 8A; $F(1,22) = 0.00$, $p = 0.96$). There was also no significant main effect of separation ($F(1,22) = 2.23$, $p = 0.14$), but there was a significant main effect of delay ($F(1,22) = 145.69$, $p < 0.001$) as well as an interaction between the two was observed ($F(1,21) = 87.12$, $p < 0.001$). Additionally, there was a significant interaction between separation distance and delay ($F(1,22) = 46.66$, $p < 0.001$). Post hoc analyses using Tukey's method revealed that the percent accuracy on trials with a 2 s delay and small stimulus separations was significantly higher than any other combination of delay and separation ($p < 0.05$). Additionally, the percent accuracy on trials with a 2 s delay and small stimulus separations was also significantly different from trials with a 6 s delay and large stimulus separation ($p < 0.05$), but was not significantly different than that of trials with a 6 s delay and small stimulus separation ($p > 0.05$). Percent accuracy on trials with a 6 s delay and a small stimulus separation was also significantly different from that on trials with a 6 s delay and large stimulus separations ($p < 0.05$). There were no significant interactions between treatment and delay (Fig. 8A; $F(1,22) = 0.03$, $p = 0.86$), treatment and separation distance ($F(1,22) = 0.00$, $p = 0.99$), or treatment x delay x separation distance ($F(1,22) = 0.02$, $p = 0.89$).

Furthermore, independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 8B; $t(22) = 1.11$, $p = 0.28$), selection trials completed (Fig. 8C; $t(22) = 0.79$, $p = 0.44$), correction trials completed (Fig. 8D; $t(22) = 0.90$, $p = 0.38$), or reward latency (Fig. 8E; $t(22) = -1.91$, $p = 0.07$).

A mixed 2x2 ANOVA (treatment, latency type) revealed no significant difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 8F; $F(1,22) = 0.65$, $p = 0.43$), but the incorrect trial latency was significantly longer than the correct trial latency ($F(1,22) = 6.23$, $p = 0.02$). There was no significant interaction between treatment and latency type ($F(1,22) = 0.48$, $p = 0.49$).

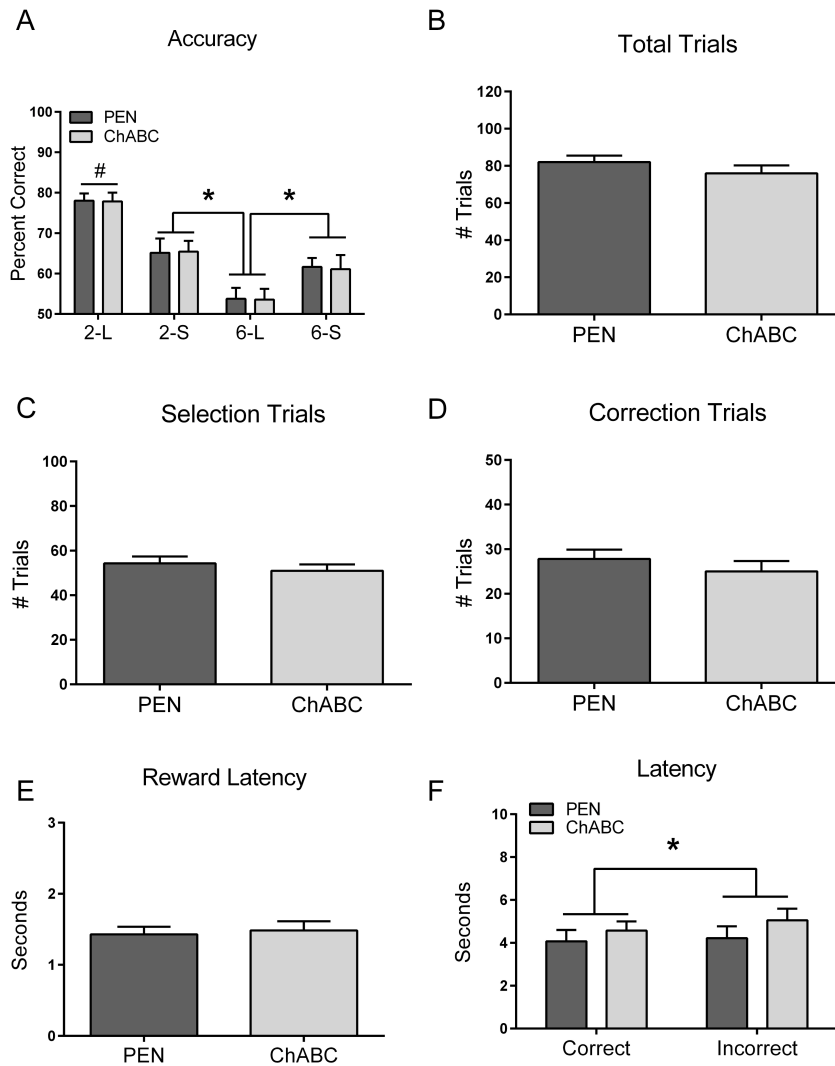


Figure 1.8: Effects of PEN or ChABC infusions into the PPC on TUNL with a variable 2 s or 6 s delay (Condition 2). All values represent the average (Mean \pm SEM) of the testing days. (A) Percent accuracy on trials with either a 2 s delay and large stimulus separation, 2 s delay and small stimulus separation, 6 s delay and large stimulus separation, and 6 s delay and small stimulus separation. ChABC had no effect on percent accuracy. Percent accuracy on trials with a 2 s delay and a large stimulus separation was significantly higher than any other group. Percent accuracies on trials with a 2 s and 6 s delay with small stimulus separations were not significantly different from one another, but were both significantly higher than percent accuracy on trials with a 6 s delay and a large stimulus separation. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on correct or incorrect latency. Incorrect latency was significantly longer than correct latency. PEN, $n = 12$; ChABC, $n = 12$; # significantly different from all other groups ($p < 0.001$); * $p < 0.05$.

ChABC infusions in the PPC had no effect on the performance of rats in condition 3 (2 s delay with a 1 s inter-trial interval)

A mixed 2x2 ANOVA (treatment, separation) revealed that the percent accuracy of ChABC-treated rats was not significantly different than that of PEN-treated rats (Fig. 9A; $F(1,22) = 0.20$, $p = 0.66$). Rats, however, did perform significantly better on large separation trials compared to small separation trials ($F(1,22) = 25.36$, $p < 0.001$), but there was no significant interaction between stimulus separation and treatment ($F(1,22) = 0.16$, $p = 0.70$). Furthermore, independent-samples t-tests revealed that ChABC-treated rats did not perform significantly differently from PEN-treated rats in terms of total trials completed (Fig. 9B; $t(22) = 1.75$, $p = 0.09$), selection trials completed (Fig. 9C; $t(22) = 1.49$, $p = 0.15$), correction trials completed (Fig. 9D; $t(22) = 0.70$, $p = 0.49$), or reward latency (Fig. 9E; $t(22) = -1.58$, $p = 0.13$).

A mixed 2x2 ANOVA (treatment, latency type) revealed no significant difference in the correct and incorrect trial latency of ChABC-treated versus PEN-treated rats (Fig. 9F; $F(1,22) = 0.11$, $p = 0.75$), but that the incorrect trial latency was significantly longer than the correct trial latency ($F(1,22) = 21.88$, $p < 0.001$). There was no significant interaction between treatment and latency type ($F(1,22) = 0.00$, $p = 0.99$).

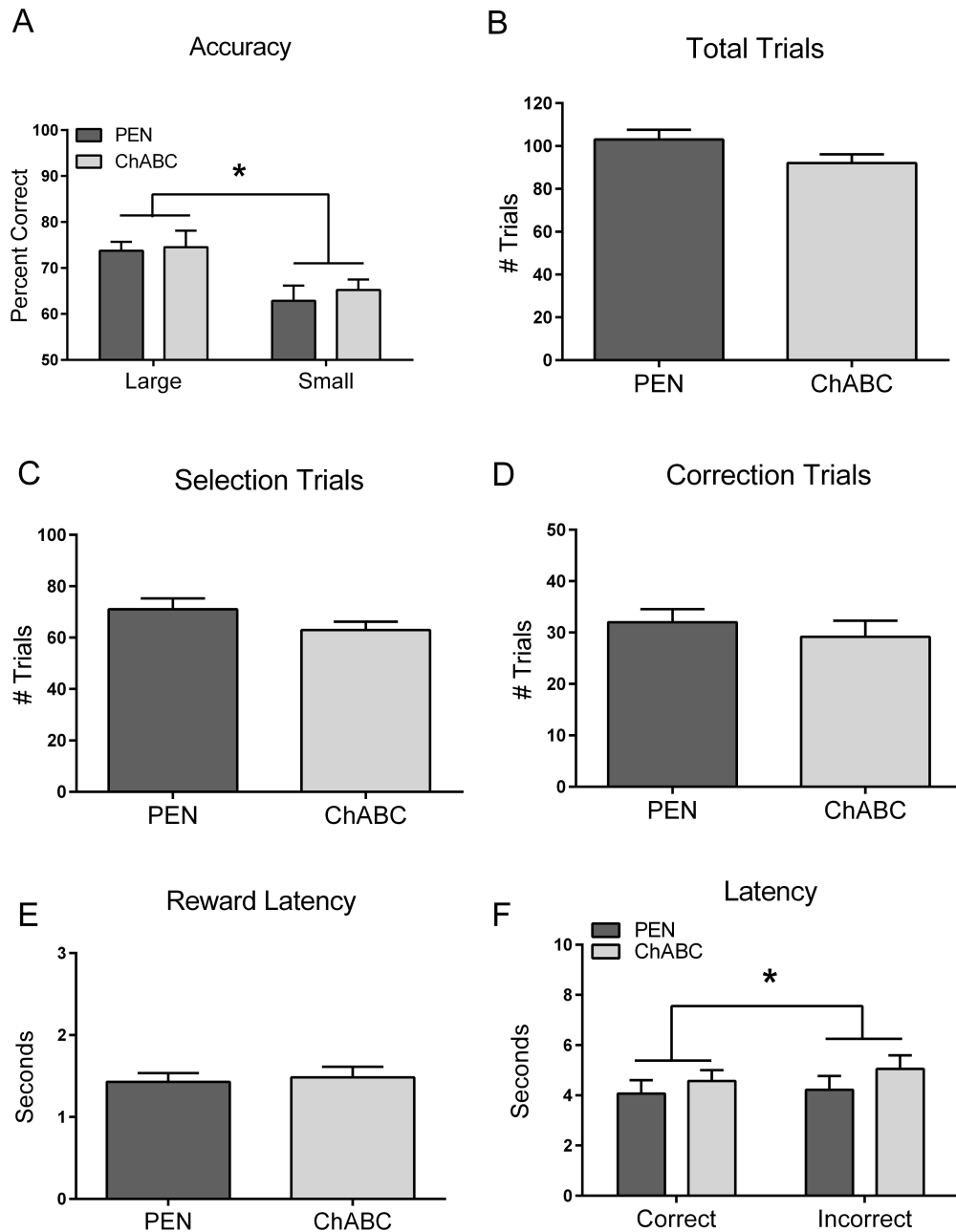


Figure 1.9: Effects of PEN or ChABC infusions into the PPC on TUNL with a 2 s delay and a 1 s ITI (Condition 3). All values represent the average (Mean \pm SEM) of the testing days. (A) ChABC had no effect on percent accuracy. Percent accuracy on trials with large stimulus separations was significantly higher than percent accuracy on trials with small stimulus separations. (B) ChABC had no effect on the total number of trials completed. (C) ChABC had no effect on the number of selection trials completed. (D) ChABC had no effect on the number of correction trials completed. (E) ChABC had no effect on reward latency. (F) ChABC had no effect on correct or incorrect latency. Incorrect latency was significantly longer than correct latency. PEN, $n = 12$; ChABC, $n = 12$; * $p < 0.05$.

ChABC infusions in the PPC had no effect on the performance of rats in condition 4 (20 s delay)

Overall accuracy, accuracy at large stimulus separations, accuracy at small stimulus separations, total trials completed, selection trials completed, and correction trials completed were analyzed with mixed 2x3 ANOVAs (treatment, testing day). Overall accuracy was not significantly different between PEN and ChABC-treated rats (Fig. 10A; $F(1,22) = 0.25$, $p = 0.62$). Additionally, there was no main effect of testing day ($F(2,44) = 0.59$, $p = 0.56$), and no interaction between treatment and testing day ($F(2,44) = 0.20$, $p = 0.82$). The percent accuracy for trials with large stimulus separations was not significantly different between PEN and ChABC-treated rats (Fig. 10B; $F(1,22) = 2.92$, $p = 0.10$). Furthermore, there was no main effect of testing day ($F(2,44) = 0.64$, $p = 0.54$) and no interaction between treatment and testing day ($F(2,44) = 0.94$, $p = 0.40$). The percent accuracy for trials with small stimulus separations was not significantly different between PEN and ChABC-treated rats (Fig. 10C; $F(1,22) = 0.93$, $p = 0.35$). There was also no main effect of testing day ($F(2,44) = 0.35$, $p = 0.71$), and no interaction between treatment and testing day ($F(2,44) = 0.28$, $p = 0.76$).

There was no difference in the number of total trials completed by PEN and ChABC-treated rats (Fig. 10D; $F(1,22) = 0.97$, $p = 0.34$). However, there was a main effect of testing day ($F(2,44) = 4.19$, $p = 0.022$), but no interaction between treatment and testing day ($F(2,44) = 0.98$, $p = 0.39$). Post hoc analyses using Tukey's method revealed that rats performed significantly more mean total trials on days 5-6 compared to days 1-2 ($p < 0.05$).

There was also no difference in the number of selection trials completed by PEN and ChABC-treated rats (Fig. 10E; $F(1,22) = 0.06$, $p = 0.81$). There was, however, a significant main effect of testing day ($F(2,44) = 9.21$, $p < 0.001$), but no interaction between treatment and testing day ($F(2,44) = 0.62$, $p = 0.55$). Post hoc analyses using Tukey's method revealed that rats performed significantly more mean selection trials on days 5-6 compared to days 1-2 ($p < 0.05$).

There was no difference in the number of correction trials completed by PEN and ChABC-treated rats (Fig. 10F; $F(1,22) = 1.52$, $p = 0.23$). There was also no significant main effect of testing day ($F(2,44) = 0.46$, $p = 0.63$) and no interaction between treatment and testing day ($F(2,44) = 0.22$, $p = 0.81$).

An independent samples t-test revealed that PEN and ChABC-treated rats did not have any significant differences in reward latency (Fig. 10G; $t(22) = -1.43$, $p = 0.17$). A mixed 2x2 ANOVA (treatment, latency type) revealed no significant difference in the correct and incorrect

trial latency of ChABC-treated versus PEN-treated rats (Fig. 10H; $F(1,22) = 0.19$, $p = 0.66$). Additionally, there was no difference between correct trial latency and incorrect trial latency ($F(1,22) = 0.14$, $p = 0.72$), as well as no interaction between treatment and latency type ($F(1,22) = 2.67$, $p = 0.12$).

When investigating the initial two days of the 20 s delay condition (testing days 10-11), independent samples t-tests revealed that there were no significant differences between PEN and ChABC-treated rats with regards to total percent accuracy (Fig. 10A; $t(22) = -0.39$, $p = 0.70$), large separation percent accuracy (Fig. 10B; $t(22) = -0.49$, $p = 0.63$), small separation percent accuracy (Fig. 10C; $t(22) = 0.50$, $p = 0.62$), total trials completed (Fig. 10D; $t(22) = 0.09$, $p = 0.93$), selection trials completed (Fig. 10E; $t(22) = -0.46$, $p = 0.65$), or correction trials completed (Fig. 10F; $t(22) = 0.58$, $p = 0.57$).

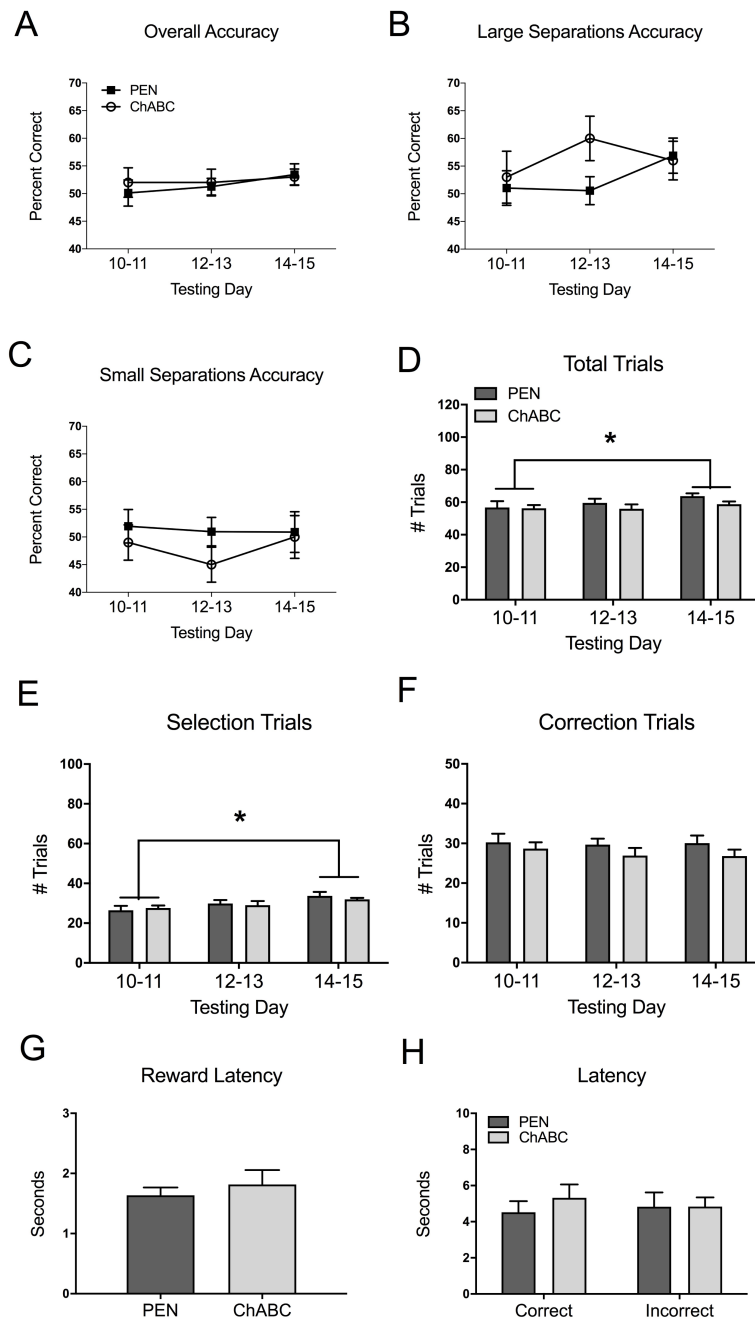


Figure 1.10: Effects of PEN or ChABC infusions into the PPC on TUNL with a delay of 20 s (Condition 4). All values represent the average (Mean \pm SEM) of the testing days. (A) ChABC had no effect on overall percent accuracy. (B) ChABC had no effect on the percent accuracy on trials with large stimulus separations. (C) ChABC had no effect on the percent accuracy on trials with small stimulus separations. (D) ChABC had no effect on the total number of trials completed. Rats performed significantly more total trials on testing days 14-15 compared to testing days 10-11. (E) ChABC had no effect on the number of selection trials completed. Rats performed significantly more selection trials on testing days 14-15 compared to testing days 10-11. (F) ChABC had no effect on the number of correction trials completed. (G) ChABC had no effect on reward latency. (H) ChABC had no effect on correct or incorrect latency. PEN, $n = 12$; ChABC, $n = 12$; * $p < 0.05$.

2.4.3 Quantification of the effects of ChABC on PNNs in the mPFC

To confirm the degradation of CSPGs and PNNs by ChABC in the mPFC, we stained with Wisteria Floribunda Agglutinin (WFA), a marker for CSPGs that preferentially labels PNNs, and mouse anti-chondroitin-4-sulfate (C4S), a marker for cleaved components of CSPGs. WFA intensity was significantly reduced within the mPFC of ChABC-treated rats compared to controls (Fig. 11D; $t(21) = 3.78$, $p = 0.001$). Staining intensity for C4S was significantly higher within the mPFC of ChABC-treated rats than in controls (Fig. 11E; $t(21) = -3.81$, $p = 0.001$). As a control reference region, the somatosensory jaw area (S1J) was assessed. In this region, there was no difference in WFA (Fig. 11D; $t(21) = 1.89$, $p = 0.07$) or C4S (Fig. 11E; $t(21) = -0.79$, $p = 0.44$) intensities compared to controls.

PNNs commonly ensheath parvalbumin containing (PV+) inhibitory interneurons in the cortex (Härtig et al., 1992). To assess whether degradation of PNNs was paired with PV+ interneuron loss, staining with an antibody specific to PV+ was performed. PNN counts (Fig. 12E; $t(21) = 3.129$, $p = 0.005$), PV+ cell counts (Fig. 12F; $t(21) = 2.39$, $p = 0.027$), and the percentage of PV+ cells surrounded by PNNs (Fig. 12G; $t(21) = 2.23$, $p = 0.037$) were all significantly reduced in ChABC-treated rats compared to controls.

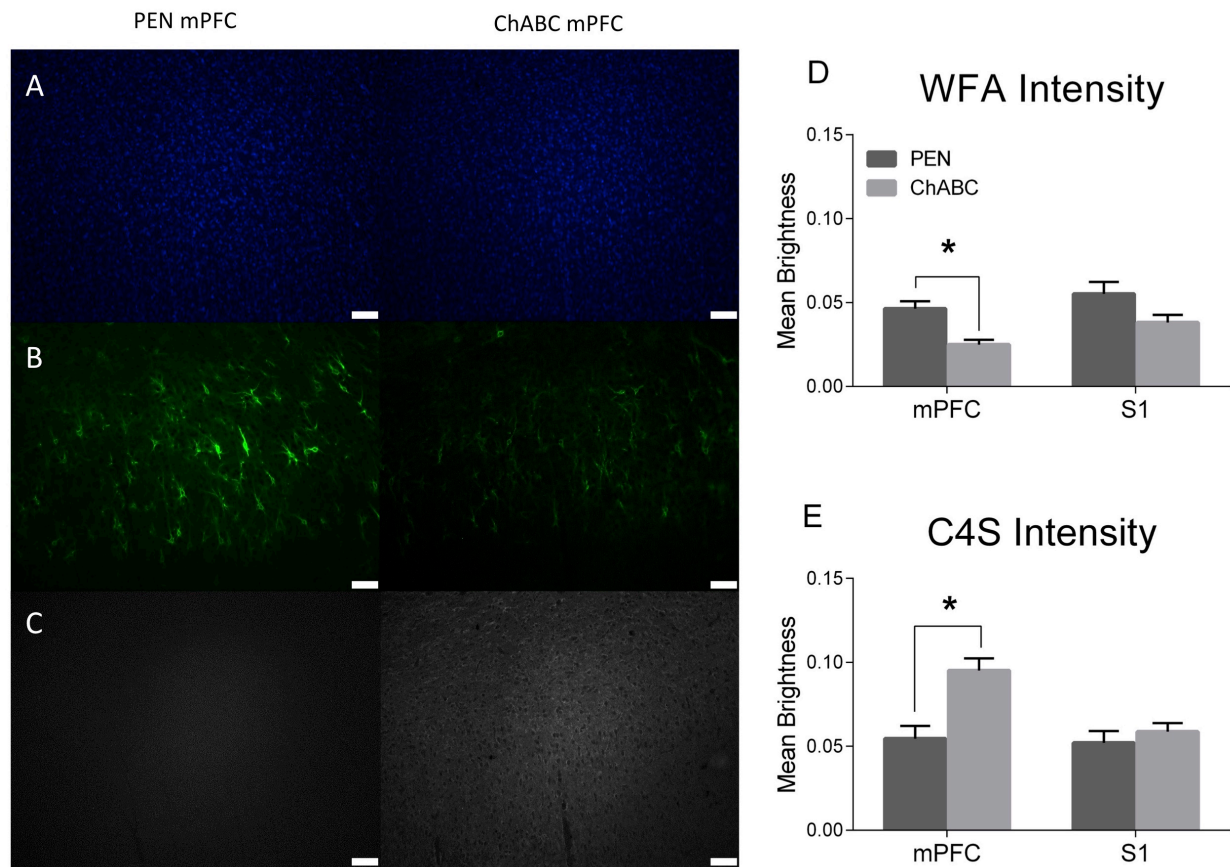


Figure 1.11: ChABC infusions into the mPFC decreased WFA expression of the extracellular matrix and increased C4S staining for cleaved CSPG stubs. Representative images captured at 10x magnification of (A) DAPI, (B) WFA, and (C) C4S. (D) ChABC infusions into the mPFC significantly reduced WFA expression in the mPFC, but not in the lateral S1 reference region. (E) ChABC infusions into the mPFC significantly increased the expression of C4S in the mPFC, but had no effect within the S1 reference region. Scale bar (white): 100 μ m; PEN, n = 12; ChABC, n = 11; *p < 0.05.

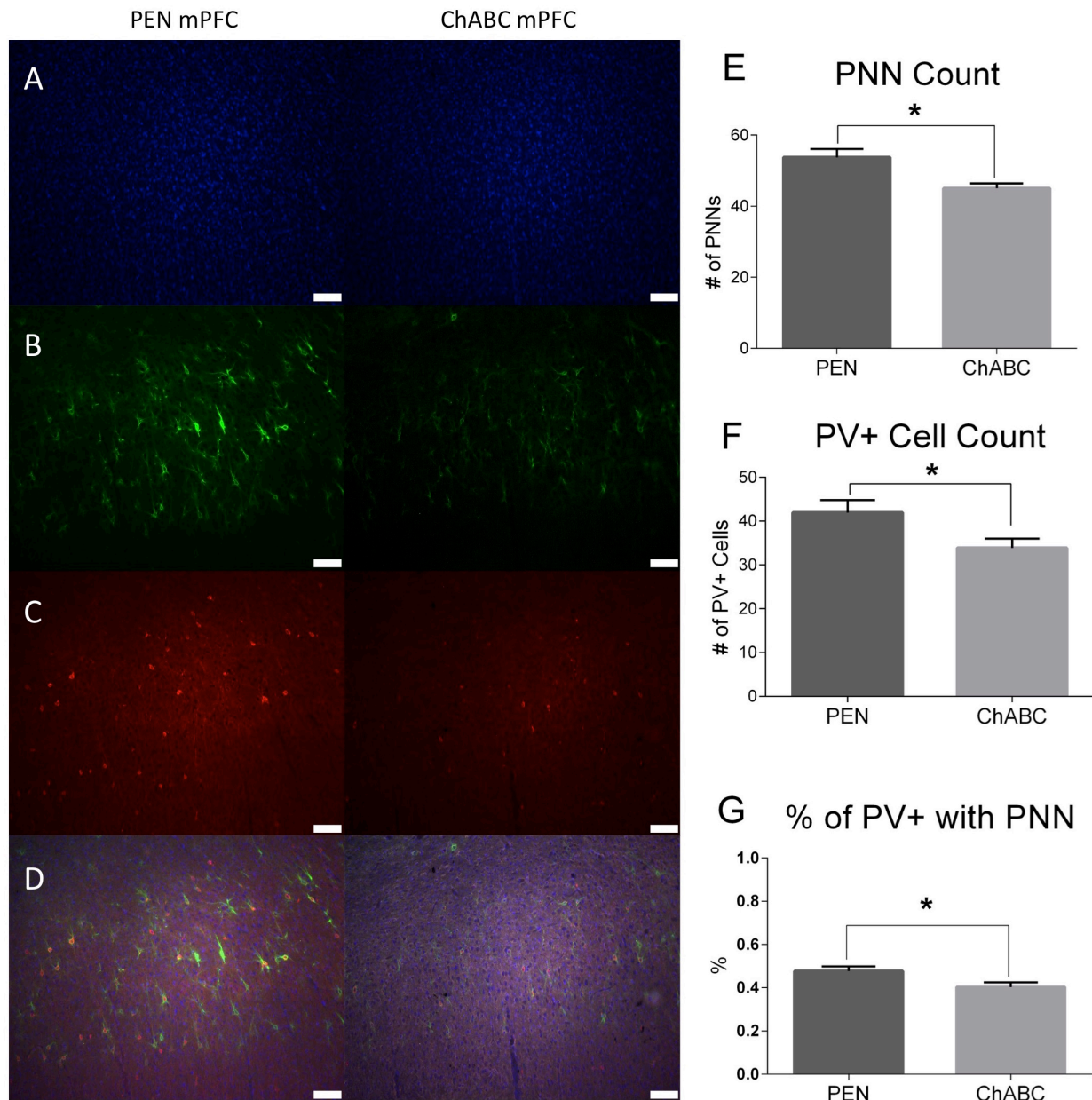


Figure 1.12: ChABC infusions into the mPFC reduced PV+ cell density but did not affect PNN density. Representative images captured at 10x magnification of (A) DAPI, (B) WFA, (C) PV+, and (D) merged images. (E) PNN count was significantly reduced following ChABC infusions (F) PV+ cell count was significantly reduced following ChABC infusions. (G) ChABC-treated animals had significantly fewer PV+ cells surrounded by PNNs compared to controls. Scale bar (white): 100µm; PEN, n = 12; ChABC, n = 11; *p < 0.05.

2.4.4 Quantification of the effects of ChABC on PNNs in the PPC

Staining intensity for C4S was significantly higher within the PPC of ChABC-treated rats than in controls (Fig. 13D; $t(22) = -2.46$, $p = 0.022$). WFA intensity, however, was not significantly different within the PPC of ChABC-treated rats compared to controls (Fig. 13E; $t(22) = 0.83$, $p = 0.42$). As a control reference region, an area lateral to the PPC was chosen. In this region, there was no difference in WFA (Fig. 13D; $t(22) = 0.43$, $p = 0.68$) or C4S (Fig. 13E; $t(22) = 0.35$, $p = 0.73$) intensities compared to controls.

Like the protocol for the mPFC, the number of PV+ cells and PNNs was also assessed in the PPC. ChABC-treated rats were not significantly different than controls in terms of PNN count (Fig. 14E; $t(21) = 0.424$, $p = 0.676$), PV+ cell count (Fig. 14F; $t(21) = -0.08$, $p = 0.94$), or the percentage of PV+ cells surrounded by PNNs (Fig. 14G; $t(21) = -0.03$, $p = 0.97$).

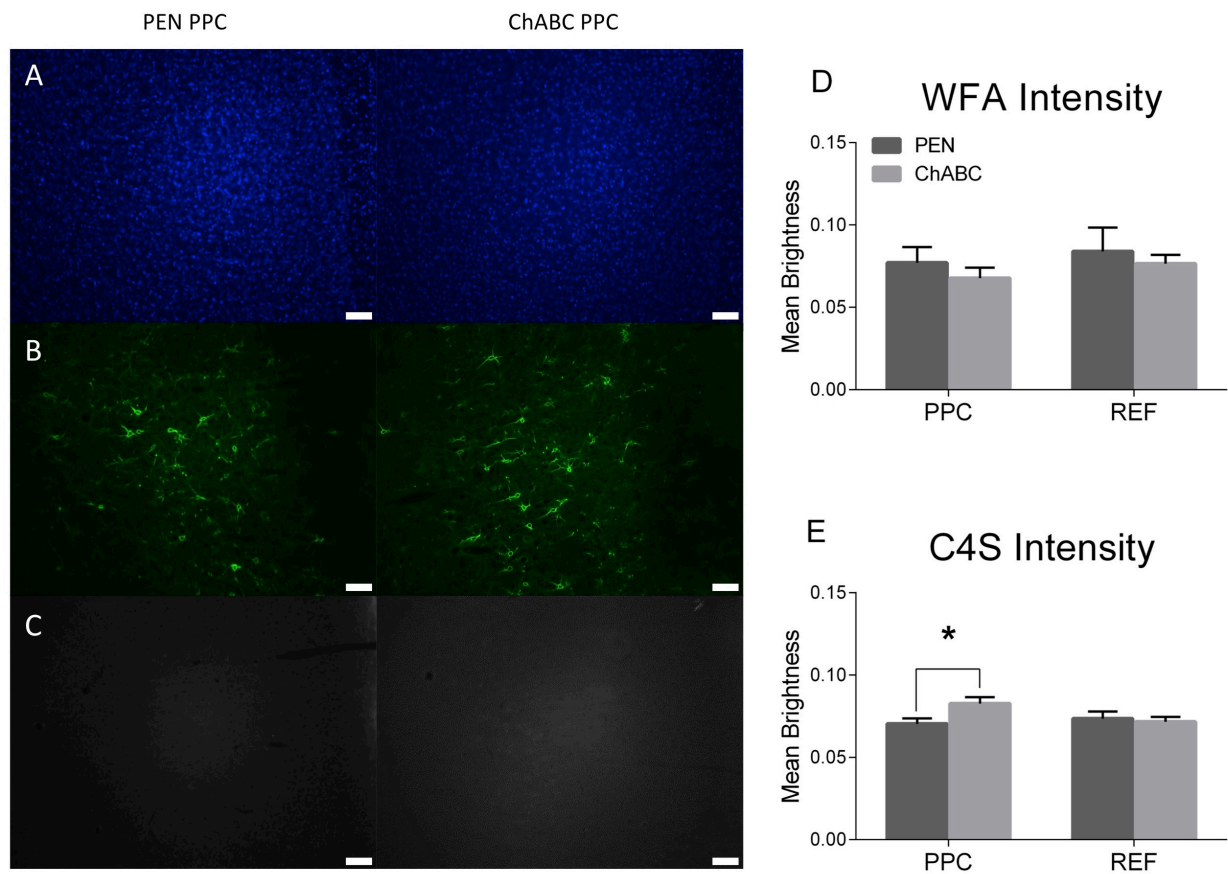


Figure 1.13: ChABC infusions into the PPC increased C4S staining for cleaved CSPG stubs but had no effect on WFA intensity. Representative images captured at 10x magnification of (A) DAPI, (B) WFA, and (C) C4S. (D) ChABC infusions into the PPC had no effect on WFA expression in the PPC or in a reference region lateral to the PPC. (E) ChABC infusions into the PPC significantly increased the expression of C4S in the PPC, but had no effect within a reference region lateral to the PPC. Scale bar (white): 100 μ m; PEN, n = 12; ChABC, n = 12; *p < 0.05.

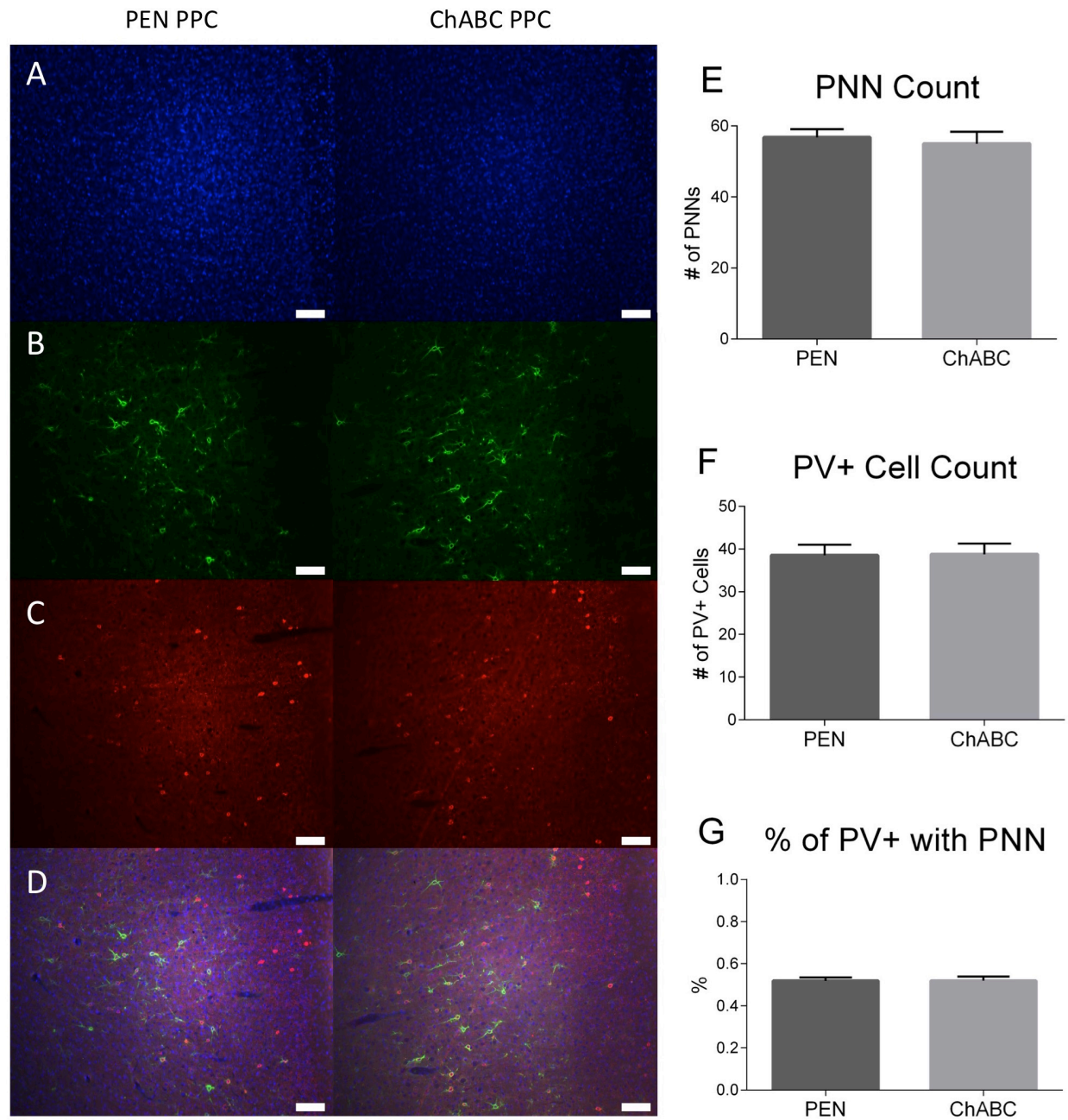


Figure 1.14: ChABC infusions into the PPC did not reduce PNN density or PV+ cell density. Representative images captured at 10x magnification of (A) DAPI, (B) WFA, (C) PV+, and (D) merged images. (E) ChABC infusions into the PPC had no effect on PNN count (F) ChABC infusions into the PPC had no effect on PV+ cell count (G) ChABC infusions into the PPC had no effect on the percent of PV+ cells surrounded by PNNs. Scale bar (white): 100µm; PEN, n = 11; ChABC, n = 12.

2.4.5 PNN regrowth in the mPFC following ChABC treatment

WFA intensity was normalized for each rat by using S1 as a control reference region. PEN-treated rats that were sacrificed 30 days after surgery had a significantly higher normalized WFA intensity than ChABC-treated rats that were sacrificed 7 days after surgery (Fig. 15; $t(14) = 3.66$, $p = 0.003$) and 30 days after surgery ($t(21) = 3.23$, $p = 0.004$). Additionally, ChABC-treated rats that were sacrificed 30 days after surgery had a significantly higher normalized WFA intensity than ChABC-treated rats that were sacrificed 7 days after surgery ($t(13) = 4.13$, $p = 0.001$).

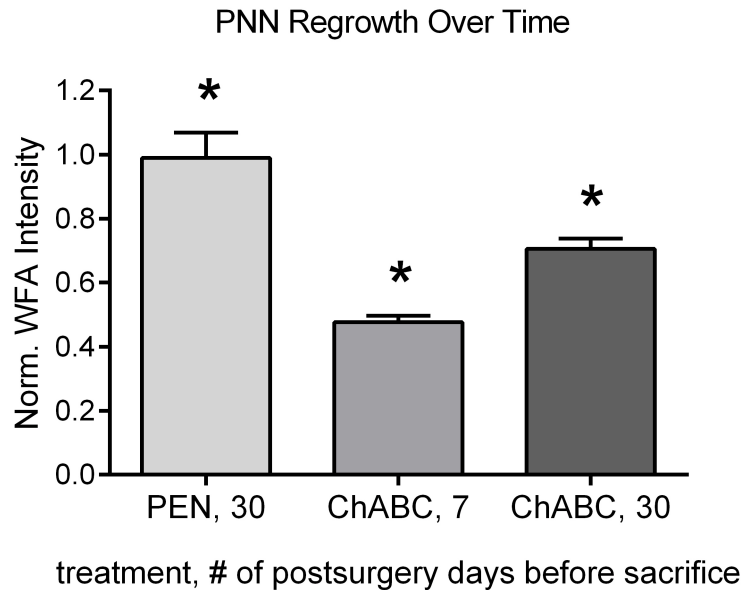


Figure 1.15: Comparison of normalized WFA intensities of rats that were sacrificed that were sacrificed 30 after mPFC PEN infusions and rats that were sacrificed 7 or 30 days after mPFC ChABC infusions. WFA intensity was normalized for each rat by using S1 as a control reference region. PEN-treated rats that were sacrificed 30 days after surgery had a significantly higher normalized WFA intensity than ChABC-treated rats that were sacrificed 7 days after surgery and 30 days after surgery. ChABC-treated rats that were sacrificed 7 days after surgery had a significantly higher normalized WFA intensity than ChABC-treated rats that were sacrificed 30 days after surgery. ChABC 7, $n = 4$; ChABC 30, $n = 11$; PEN 30, $n = 12$; * significantly different from all other groups ($p < 0.05$).

2.5 Discussion

Targeted delivery of ChABC into the mPFC and PPC was performed to degrade CSPGs and PNNs prior to rats being tested on the TUNL task. ChABC infusions into the mPFC initially led to a higher percent accuracy, more selection trials completed, and fewer correction trials when a novel 20 s delay (condition 4) was introduced in the TUNL task. ChABC infusions into the mPFC had no effect on a standard 6 s delay (condition 1), a variable 2 s or 6 s delay (condition 2), or an interference condition (condition 3) in TUNL. ChABC infusions into the PPC resulted in no effect in any of the 4 TUNL conditions. Notably, immunohistochemistry revealed that ChABC infusions into the mPFC increased C4S intensity and decreased WFA intensity. Furthermore, ChABC significantly reduced PNN count, PV+ cell count, and the percent of PV+ cells surrounded by PNNs. In the PPC, ChABC infusions led to increased C4S intensity, but had no effect on WFA intensity, PNN count, PV+ cell count, or the percent of PV+ cells surrounded by PNNs.

In the present study, stereotaxic coordinates and ChABC volumes for mPFC infusions were based on our previous study (Paylor et al. 2018). Like our previous study, mPFC infusions of ChABC resulted in significantly increased C4S intensity, indicative of more CSPG stubs, and decreased WFA intensity, indicative of fewer intact CSPGs in the ECM. In both the present study and our previous study, there was a significant reduction in PNNs following ChABC infusions (Paylor et al. 2018). There was also a reduction in PV+ cells in the present study, however PV+ cell counts were unaffected in our previous study. PNNs protect the neurons that they ensheath from oxidative stress and support functional cell signaling and ion buffering (Morawski et al. 2004; Brückner et al. 1993; Dzyubenko et al. 2016). Consistent with this, PNN degradation in the present study may have impaired the physiological function of PV+ interneurons that they surrounded, which could have led to PV+ cell loss (Morishita et al. 2015; Chu et al. 2018). Alternatively, the experience dependent plasticity of PV+ cell networks could explain why there was a PV+ cell reduction in the present study but not our previous study (Donato et al. 2013). While the present study tested rats in the TUNL task, our previous study tested rats using a battery of tasks including pre-pulse inhibition, set-shifting, reversal learning, cross-modal object recognition, and oddity (Paylor et al. 2018). The difference in tasks performed between the two studies could have led to differences in PV+ cell networks, which

could explain why there was a reduction in PV⁺ cells in the present study, but not our previous study (Donato et al. 2013).

ChABC infusions into the PPC had no effect on PNN count. The reduction in mPFC PNNs but not PPC PNNs in the present study could be the result of larger ChABC spread in the PPC, since it has a wider coronal section than the mPFC. A lack of PNN reduction in the PPC may also have resulted from infusions being done anterior to the PPC. Infusions were performed between -3.30 and -3.80 mm posterior to bregma, while the PPC was imaged between -3.84 mm and -4.68 mm posterior to bregma. Alternatively, it is possible that PNNs in the PPC regrew at a faster rate than PNNs in the mPFC. As an example of different brain regions having different rates of PNN regrowth, it appears that PNNs in the V1 regrow more quickly than PNNs in the mPFC. One group targeted the primary visual cortex (V1) and infused 3.1 μ L of ChABC into each hemisphere (Lensjø et al. 2016). They found that WFA intensity in the V1 (after being normalized to a reference region) went from about 0.05 seven days post surgery to about 0.6 thirty days post-surgery (Lensjø et al. 2016). In the present study, we injected 0.6 μ L of ChABC into each mPFC hemisphere, which led to a mPFC normalized WFA intensity of about 0.45 seven days post surgery to about 0.7 thirty days post surgery. This brain region-dependent PNN regrowth rate may explain why PNNs in the mPFC, but not the PPC, were significantly reduced in the present study.

The battery of TUNL conditions in this study was designed to assess the effects that PNN degradation has on spatial WM, susceptibility to interference, and behavioural flexibility. Interestingly, in a single 60-minute block of TUNL with a variable 2 s or 6 s delay (condition 2), rats performed worst on trials with a 6 s delay and large stimulus separations (Fig. 4A, 8A). Conversely, in previous experiments that used constant 2 s and 6 s delays in counterbalanced 30-minute blocks, rats performed worst on trials with a 6 s delay and small stimulus separations (Hurtubise et al. 2017; Davies et al. 2017). This difference implies that rats change their strategy in TUNL when tested on a variable versus a constant delay. In general, rats performed better on trials with large stimulus separations compared to trials with small stimulus separations. This result is consistent with previous studies, and was expected because decreased separation distances between stimuli challenge pattern separation more (Davies et al. 2016; Hurtubise et al. 2017; Scott et al. 2019). Additionally, in general, rats performed better on trials with shorter delays compared to trials with longer delays. This too, was expected because increased delays in

TUNL challenge WM more. The response latency of rats on incorrect trials was longer than the response latency on correct trials, which implies that rats hesitate when unsure of the correct choice.

The results from the present study support the idea that cortical PNNs play a subtle role in complex cognitive tasks. Rats treated with mPFC ChABC infusions had initially improved percent accuracy, performed more selection trials, and performed fewer correction trials compared to controls in the 20 s delay condition. The initial improvement in percent accuracy was specific to large stimulus separation trials, and did not occur in small stimulus separation trials. This is likely because small stimulus separation trials at a 20 s delay are very challenging, and difficult for a rat to perform above chance. The performance of more selection trials can be generally attributed to more correct choices being made and lower response latencies. The performance of fewer correction trials represents less perseveration on incorrect choices, and can be inferred as increased behavioural flexibility (Kumar et al. 2015; Lins et al. 2015; Hurtubise et al. 2017). This initial improvement when a 20 s delay was used, but not when 2 s or 6 s delays were used, represents a delay-dependent effect, which has previously been observed following mPFC manipulation (McAllister et al. 2013; Delatour and Gisquet-Verrier 1999; Herremans et al. 1996). Perhaps the improved performance of ChABC-treated rats was related to a period of increased synaptic plasticity caused by ChABC, which could have facilitated the functional connections of neurons that are involved in WM. Memory improvements coupled with increased synaptic plasticity have previously been observed in animals with attenuated PNNs (Carulli et al. 2010; Morellini et al. 2010; Romberg et al. 2013). Alternatively, the improvement in initial performance could be the result of increased behavioural flexibility, because the 20 s delay condition was the only condition that introduced a novel delay. Interestingly, one group observed a similar effect following mPFC lesions. When they trained rats on a 5 s delay, mPFC lesions impaired the performance of rats when tested on a novel 20 s delay (Delatour and Gisquet-Verrier 1999). However, mPFC-lesioned rats that were trained on a variable 0, 5, 10, or 20 s delay were not impaired when switched to a non-variable 20 s delay (Gisquet-Verrier et al. 2000). The initial improved performance in the 20 s delay condition could also be explained by the inability of rats to wait for a stimulus, as this has previously been observed following mPFC inactivation (Narayanan et al. 2006). Consistent with this, results indicated a general trend towards longer response latencies in ChABC-treated rats compared to controls in all conditions

tested. Finally, improved performance following mPFC ChABC, but not PPC ChABC infusions was surprising, because WM has been proposed to rely on fronto-parietal circuits (Eriksson et al. 2015). Perhaps this dissociation is the result of the mPFC and PPC contributing to spatial WM in different ways.

In conclusion, this experiment showed that modulation of PNNs in the mPFC results in subtle consequences for adapting to a novel delay in the TUNL spatial WM task, while PNNs in the PPC do not. Automated touchscreen tasks such as TUNL are useful tools because they allow for careful control over task difficulty, which can reveal subtle behavioural effects associated with PNN reductions. Reduced cortical PNNs have been observed in patients with CNS disorders, as well as animal models of CNS disorders. This makes ChABC infusions a valuable tool for studying the relationship between PNN reductions and the cognitive deficits seen in CNS disorders, because it can acutely degrade PNNs in specific brain areas. However, surgical infusions of ChABC are labor intensive and there are issues with these surgeries being done repeatedly throughout a rat's lifespan. Recently, an inducible viral vector has been developed that can efficiently and reversibly degrade PNNs by regulated delivery of ChABC (Burnside et al. 2018). In future studies, this technology can be used to carefully control PNN density, and can be paired with behavioural tasks to study the relationship between PNN density and cognitive function.

2.6 Materials and Methods

2.6.1 Subjects:

Forty-eight Long Evans rats were trained on the TUNL task in two separate cohorts. All animals were housed in clear, ventilated plastic cages in a temperature-controlled vivarium. Each subject was provided with a plastic tube for enrichment, and was maintained on a 12h:12h light-dark cycle. All experimental procedures were conducted during the light phase, and subjects were left undisturbed during the dark phase. Animals were food restricted and were maintained at 85% of their free-feeding weight. Water was available *ad libitum*, except during testing. All experiments were approved by the University of Saskatchewan Research Ethics Board and were conducted in accordance with the standards of the Canadian Council on Animal Care.

2.6.2 Training apparatus:

All training and testing took place in eight touchscreen-equipped operant conditioning chambers (Lafayette Instruments, Lafayette, IN, USA). These chambers are trapezoidal in shape, with a food magazine port positioned parallel and across from the wall with the touchscreen. A black polycarbonate mask with 14 squares presented in a 7x2 pattern (7 squares horizontally, 2 squares vertically) covered the touchscreen during the TUNL task. This pattern was visually obstructed with the use of a spring-loaded “response shelf” that rats needed to intentionally press down in order to nose-poke the touchscreen. On the opposite side of the chamber, the food magazine dispensed odorless reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, Frenchtown, NJ). This food magazine was also equipped with a reward light and an infra-red nose-poke detector. A metal mesh with holes constituted the floor of the chamber, and the roof was a transparent plastic lid. Each operant conditioning chamber was located on a sliding shelf at the base of a sound-attenuating large wooden box. In^[1]_{SEP} addition to the chamber, each box contained a pellet dispenser, video camera, small ventilation fan, and a house light that was activated following incorrect responses.

2.6.3 Handling and habituation:

Upon being transported to the vivarium, rats were left undisturbed for a minimum of two weeks. After this time, they were each handled for two consecutive days prior to when training began. Each rat was handled in the touchscreen rooms to familiarize them to that environment. To get to this room from the vivarium, rats were transported up an elevator with the use of a cart. After becoming accustomed to this transportation route and handling for three days, rats were habituated to the touchscreen box. On the first day of habituation, each rat was assigned to and placed into a specific touchscreen chamber for one hour. During this time, ten reward pellets were manually placed into the touchscreen’s magazine port, and all technology was turned on (2 computers, each controlling 4 touchscreen devices).

2.6.4 TUNL pretraining:

The pretraining protocol followed a modified version of the instructions provided by

Lafayette, which involved completion of a series of stages until criterion was reached. TUNL pretraining was composed of four stages: initial touch, must touch, must initiate, and punish incorrect. Initial Touch Training introduced the rats to the touchscreen stimuli and their relationship to a food reward. During each trial, 1 of the 14 squares was illuminated. When a rat touched the illuminated square, 3 reward pellets were immediately dispensed into the food port. If the illuminated square was left untouched for 30 seconds, however, the stimulus was removed and only a single pellet was dispensed. Each trial was followed by an ITI period of 20 seconds. In order to reach criterion for Initial Touch Training, rats must have completed 100 trials in 60 minutes. During each trial of Must Touch Training 1 of the 14 squares were illuminated, but they remained lit until touched by a rat, upon which time a single reward pellet was dispensed. Criterion for this stage involved completing 100 trials in 60 minutes. During Must Initiate Training, rats are required to poke their nose in the food magazine to initiate trials identical to those in Must Touch Training. Criterion involved completing 100 trials in 60 minutes. The final pretraining stage was Punish Incorrect Training, in which each trial began with a rat poking its nose into the food reward port. This led to the presentation of a stimulus (illuminated square). If a rat touched the stimulus, a reward pellet was dispensed and a new trial began following an ITI. If the rat touched an unilluminated square, however, a timeout period began. During the timeout, no reward pellet was dispensed, the house light turned on for 5 seconds, and then an ITI began. The previous trial was then repeated until the rat correctly selected the correct stimulus (these repeated trials were termed as ‘correction trials’). Criterion was the completion of 100 trials within 60 minutes with >80% accuracy on two consecutive days.

2.6.5 TUNL task acquisition:

After a rat completed pretraining, it was moved on to learn the standard TUNL task (Fig. 2). Each trial began by a rat poking its nose into the reward magazine. This initiated the sample phase, in which 1 of the 14 squares was lit. If a rat touched one of these illuminated squares, there was a 1 in 3 chance of a pellet being dispensed, and the stimulus was removed from the screen for a 2 second delay. Following the delay, the rat was required to poke its nose into the reward magazine to start the choice phase. During this phase, the sample square and a novel square were illuminated simultaneously. A correct response was made when the rat touched the novel square (non-matched to the sample square). Correct responses led to a reward pellet being

dispensed, followed by a 20 second ITI. A new 'selection trial' began at the end of the ITI, with a new square being illuminated that had to be different from the previous trial's stimulus. If an incorrect response was made, with the rat selecting the sample square, the house light turned on for 5 seconds and no reward was dispensed. After this timeout period, a 20 second ITI would begin, followed by a trial identical to the previous trial. Trials that repeat the same illuminated sample and choice square stimuli are termed 'correction trials', and are repeated consecutively until a correct choice is made. Accuracy was recorded automatically as a measure of the percent of correct responses made during correction trials. During this TUNL acquisition phase, rats were required to complete 42 trials in 30 minutes. Once the majority (>50%) of rats reached this criterion, all rats were simultaneously moved onto the final 6-second delay TUNL task (from the 2s TUNL acquisition task). In this final TUNL task, rats were trained until they completed a minimum group average of 50 trials at 65% accuracy.

2.6.6 mPFC infusions of PEN or ChABC:

Twenty-four rats were assigned to the mPFC condition, with twelve receiving PEN treatment and twelve receiving ChABC treatment. Before the operation, injectors were made from 35Ga silica tubing (WPI, Sarasota, FL), glued to PE-50 tubing, and then set up with the stereotaxic apparatus. Prior to and throughout the procedure, rats were anesthetized with an isoflurane gas inhalant (Janssen, Toronto, ON). All rats were administered a 0.5mg/kg subcutaneous dose of the analgesic Anafen (Merial Canada Inc, QC) prior to surgery. Once anesthetized, animals were positioned in the stereotaxic apparatus. Next, the scalp was cut with a scalpel and retracted to expose the skull. Holes were then drilled into the skull and the injectors set up with stereotaxic apparatus were inserted bilaterally at the following coordinates: anteroposterior (AP) +3.0 mm; lateral (L) 0.7 mm; dorsoventral (DV) 4.4 mm relative to bregma. From there, either ChABC (100 units/mL) or PEN (100 units/mL) (depending on the treatment group) was infused at the DV coordinates -4.4 mm, -4.2 mm, and -3.9 mm. These infusions were performed at a rate of 0.3 μ L per minute for 2 minutes (total infusion volume of 0.6 μ L/site). Injectors were left in place for an additional 6 minutes to allow for diffusion of the solution away from the final infusion site (DV= -3.9mm). After this time the injectors were slowly removed, the holes in the skull were filled with bone wax, and then the wound was closed with stitches.

2.6.7 PPC infusions of PEN or ChABC:

Twenty-four rats were assigned to the PPC condition, with twelve receiving PEN treatment and twelve receiving ChABC treatment. Surgery set-up and anesthetization of animals matched that of mPFC infusions. Once anesthetized, animals were positioned in the stereotaxic apparatus. Next, the scalp was cut with a scalpel and retracted to expose the skull. Holes were then drilled into the skull and the injectors set up with stereotaxic apparatus were inserted bilaterally at an anterior location and a posterior location. The anterior location had the following coordinates: AP -3.30 mm; L 2.80 mm; DV -1.20 mm relative to bregma. The posterior location had the following coordinates: AP -3.80 mm; L 3.00 mm; DV -1.30 mm relative to bregma. From there, either ChABC (100 units/mL) or PEN (100 units/mL) (depending on the treatment group) was infused at the anterior location with the DV coordinates of -1.10 mm and -1.00 mm, and at the posterior location with the DV coordinates of -1.30 mm and -1.20 mm. These infusions were performed at a rate of 0.3 μ L (anterior) or 0.6 μ L (posterior) per minute for 2 minutes (total infusion volume of 0.6 μ L/side at the anterior location and 1.2 μ L/side at the posterior location). Injectors were left in place for an additional 6 minutes to allow for diffusion of the solution away from the final infusion site (anterior DV= -1.0 mm, posterior DV= -1.20 mm). After this time the injectors were slowly removed, the holes in the skull were filled with bone wax, and then the wound was closed with stitches.

2.6.8 TUNL testing protocol:

Testing was performed between 5 to 10 days after surgery. The first 3 days (testing days 1-3) of testing consisted of a standard 6 s delay TUNL task. This tested the effect of ChABC on the same condition that rats were trained on. The next 4 days of testing (testing days 4-7) consisted of a variable 2 s or 6 s delay to test delay-dependent changes in WM. The following 2 days (days 8-9) tested for interference, in which the delay was set to 2 s and the ITI period was set from 20 s to 1 s. Finally, the final 6 days of testing (testing days 10-15) consisted of a challenging and novel 20 s delay period. The amount of testing days in each condition was based on the average number of selection trials performed per day within that condition. Each testing condition consisted of a total of 150-300 selection trials. One rat from the mPFC ChABC group was excluded as an outlier in each of the 4 conditions, as the number of selection trials that they completed in each condition was greater than 2 standard deviations from the mean.

2.6.9 Tissue collection:

After behavioural testing, rats were deeply anesthetized with isoflurane. Once fully anesthetized, they were perfused with PBS followed by 4% paraformaldehyde using infusion pumps. Following perfusion, brains were extracted and stored in a sucrose azide solution (30% sucrose, 0.1% azide in a water solvent) at 20°C. Next, the brains were mounted onto a chuck with optimal cutting temperature (OCT) gel and frozen using cold, pressurized carbon dioxide gas. They were then sectioned using a sliding microtome at 40µm and collected in a bath of 0.05M PBS.

2.6.10 Immunohistochemistry:

Slices were stored on slides in a -20-degree freezer until they were removed and thawed to room temperature (20 mins) prior to the immunohistochemistry protocol. Slides were washed 3 times in 1X PBS for 10 mins each. After this, slides were incubated with 10% Protein Block, serum-free (Dako) in 1X PBS for 1 hour. Slides were then incubated overnight with a primary antibody solution of 1% Protein Block, 1% Bovine Serum Albumin, and 98% 1X PBS with 0.1% Triton X-100 detergent. Primary antibodies used were as follows: mouse anti-chondroitin-4-sulfate (C4S; 1:400; Millipore), Wisteria Floribunda Agglutinin (WFA; 1:1000; Swant), rabbit anti-Parvalbumin (PV; 1:1000; Swant). The following day, slides were again washed 3 times, twice in PBS 1X with 1% Tween-20 and then once in PBS 1X. Slides were then incubated for 1 hour with secondary antibodies as follows: donkey anti-mouse Alexa Fluor 488 (1:200; Invitrogen), donkey anti-rabbit Alexa Fluor 546 (1:200; Invitrogen), and Streptavidin 647 (1:200; Invitrogen). After the 1-hour secondary incubation, slides were washed three times, twice in PBS 1X with 1% Tween-20 and once with PBS 1X. Slides were then labelled with 4',6-diamidino-2-phenylindole (DAPI) in Vectashield mounting medium (Vector Labs) and a cover slip was placed over the slide.

2.6.11 Widefield Epifluorescence Microscopy:

Images of sections were acquired on a LEICA DMI6000B Microscope using LAS AF computer software. To landmark the mPFC and PPC we used The Rat Brain in Stereotaxic Coordinates and selected based on features identified in the DAPI nuclear staining pattern (Paxinos & Watson 2007). The mPFC was imaged between +2.76 mm and +3.24 mm anterior to

bregma with the imaging window extending from the midline through cortical layers 1-6. All images for analysis were captured at 10X magnification and 6 images were taken per animal. Images from the somatosensory cortex were taken from within the same sections as images for the mPFC (+2.76 mm to +3.24 mm), to control for any variability between animals in staining intensity. The PPC was imaged between -3.84 mm and -4.68 mm posterior to bregma with the imaging window extending from the dorsal surface of the brain through cortical layers 1-6. Within the LAS AF software, a constant gain, exposure, and light intensity was used across all animals.

2.6.12 Image Analysis:

Tissue analysis was conducted on unmodified images by an experimenter blind to the experimental conditions. Staining intensity was quantified from all stains using the automated quantification software CellProfiler (Lamprecht et al. 2007) Images were loaded into the software and analyzed using the MeasureImageIntensity module. Cell numbers were counted manually using the ImageJ Cell Counter function.

2.6.13 Statistical Analyses:

All data are presented as mean \pm SEM. Statistical analyses were conducted in SPSS (IBM SPSS Statistics 25) using two-tailed independent-samples t-tests and mixed ANOVAs with repeated measures. Significance was set at $p < 0.05$.

3.0 GENERAL DISCUSSION

In the following section, I will discuss outstanding issues and details that were not included in the manuscript. Notably, I will also assess how the TUNL task measures the WM of rodents, and whether TUNL for rodents is relatable to human measures of WM. Additionally, based on the results from the current study, I will propose ideas and directions for future research.

3.1 The Performance of Rats in TUNL With a Constant Versus Variable Delay

An interesting result was observed in TUNL with a variable 2 s or 6 s delay (condition 2) when either the mPFC or the PPC was targeted. In this condition, both PEN and ChABC-treated rats performed best on trials with a 2 s delay and large stimulus separations and worst on trials with a 6 s delay and large stimulus separations (Fig. 4A, 8A). Conversely, in previous experiments that used constant 2 s and 6 s delays in counterbalanced 30-minute blocks, rats performed best on trials with a 2 s delay and large stimulus separations, but worst on trials with a 6 s delay and small stimulus separations (Hurtubise et al. 2017; Davies et al. 2017). In theory, trials with a 6 s delay and small stimulus separations are the most difficult because they challenge both spatial WM and spatial pattern separation the most. Therefore, it is logical that when rats performed TUNL with constant 2 s and 6 s delays in counterbalanced 30-minute blocks, they performed best on these types of trials. Perhaps that when tested on a variable 2 s or 6 s delay, rats expected a shorter 2 s delay during 6 s delay trials, which could have led them to approach the response shelf prematurely. If they habitually approached the same area of the response shelf where the stimulus was presented in the sample phase of TUNL, this would make them more likely to choose the incorrect choice on 6 s delay trials with large stimulus separations.

3.2 PV+ Cell Loss Following ChABC Infusions into the mPFC

There was a reduction in PV+ cells in the present study, however PV+ cell counts were unaffected in a previous study from our lab (Paylor et al. 2018). It is possible that PNN degradation in the present study may have impaired the physiological function of PV+ interneurons that they surrounded, which could have led to PV+ cell loss (Morishita et al. 2015; Chu et al. 2018). PNNs provide a negatively charged polyanionic microenvironment around the

neurons that they surround, which can aid in ion buffering (Brückner et al. 1993). Additionally, there is evidence that PNNs protect the neurons that they ensheath from oxidative stress (Morawski et al. 2004). For example, an inverse relationship has been discovered between the degree of oxidative stress displayed within PV+ neurons and the robustness of the PNN that surrounds them (Cabungcal et al. 2013). Additionally, key link proteins in PNNs such as aggrecan and tenascin-R help to protect neurons from iron-induced oxidative stress and neurodegeneration (Suttkus et al. 2014). It is possible that PNN degradation led to an accumulation of reactive oxygen species in PV+ neurons, which in turn could have led to either unregulated or apoptotic cell death (Morawski et al. 2004; Suttkus et al. 2014; Cabungcal et al. 2013).

Perhaps there is a temporal delay between PNN degradation and PV+ cell loss, which is an alternative explanation to the significant reduction of PV+ cells in the present study, but not in the previous study from our lab (Paylor et al 2018). Rats in the present study were sacrificed approximately 30 days after ChABC infusions, whereas in our lab's previous study they were sacrificed approximately 20 days after ChABC infusions (Paylor et al. 2018). PV+ cell loss may have occurred between 20-30 days post surgery. Alternatively, the experience dependent plasticity of PV+ cell networks could explain why there was a PV+ cell reduction in the present study but not the previous study (Paylor et al 2018). While rats were tested in the TUNL task in the present study, rats were tested using a battery of tasks including pre-pulse inhibition, set-shifting, reversal learning, cross-modal object recognition, and oddity in the previous study (Paylor et al. 2018). The difference in tasks performed between the two studies could have led to differences in PV+ cell networks, and theoretically could explain the discrepancy of PV+ cell loss between the two studies (Donato et al. 2013).

3.3 Interpretation of the Improved Performance in TUNL with a 20 s delay Following ChABC Infusions into the mPFC

There are many reasons that could explain the improved initial performance of mPFC ChABC-treated rats in the 20 s delay condition. For example, perhaps this improvement was related a period of increased synaptic plasticity caused by ChABC, which may have facilitated the functional connections of neurons that are involved in spatial WM. Memory improvements coupled with increased synaptic plasticity have previously been observed in animals with

attenuated PNNs (Carulli et al. 2010; Morellini et al. 2010; Romberg et al. 2013). For example, long-term potentiation of excitatory synapses on pyramidal neurons has been observed following PNN degradation (Carstens et al. 2016).

Alternatively, the improvement in initial performance following ChABC into the mPFC could be the result of increased behavioural flexibility, because the 20 s delay condition was the only condition that introduced a novel delay. Every other condition used a 2 or 6 s delay period, which rats were habituated to during training. ChABC-treated rats also performed fewer correction trials than controls, which is indicative of increased behavioural flexibility (Scott et al. 2019; Kumar et al. 2015). In support of this theory, one group degraded PNNs in the cortex with hyaluronidase, which resulted in enhanced flexibility in reversal learning (Happel et al. 2014).

The initial improved performance in the 20 s delay condition following ChABC infusions into the mPFC could also be explained by the inability of rats to wait for a stimulus, as this has previously been observed following mPFC inactivation (Narayanan et al. 2006). The mPFC of rats were reversibly inactivated by muscimol, which caused a higher percentage of premature responses on trials relative to controls. Interestingly, during the recovery period following the reversible inactivations, rats no longer had a higher percentage of premature responses. In the present study, the response latencies in ChABC-treated rats were generally longer compared to controls in all mPFC TUNL conditions tested. Perhaps ChABC infusions reduced rushed responding in the 20 s delay condition of the TUNL task, which could have led to the improvement in percent accuracy, selection trials, and correction trials seen in the present study.

3.4 Why Do PNNs in the mPFC, and not the PPC Appear to Play a Role in TUNL?

Improved performance following mPFC ChABC, but not PPC ChABC infusions was surprising, because WM has been proposed to rely on fronto-parietal circuits (Eriksson et al. 2015). With that being said, multiple tracts relay information between these brain regions. Perhaps the dissociation in TUNL performance following either mPFC or PPC PNN degradation was due to PNNs in these areas being differentially involved in spatial WM. In support of this, one group found that lesions to the mPFC, but not the PPC, impaired the performance of rats in a nonmatching to position task that tests spatial WM (Kolb et al. 1994). Alternatively, perhaps the dissociation in TUNL performance between PNN degradation in the mPFC and PPC was due to differing distributions of PNNs in these regions. PNNs in different brain regions preferentially

ensheathes specific subtypes of neurons. For example, PNNs in the mPFC mainly ensheathes PV+ GABAergic interneurons, whereas the CA2 region of the hippocampus has a dense expression of PNNs around excitatory pyramidal neurons (Carstens et al. 2016; Paylor et al. 2018). Based on data from the current study, it appears that PNNs in the PPC associate more commonly with PV+ cells than PNNs in the mPFC (Fig. 12G, 14G). There is evidence that the degradation of PNNs surrounding PV+ inhibitory neurons leads to increased excitability among fast spiking neurons coupled with increased synaptic plasticity (Balmer 2016; Pizzorusso et al. 2002). Conversely, the degradation of PNNs surrounding excitatory pyramidal neurons appears to suppress the plasticity of excitatory synapses (Carstens et al. 2016). Therefore, the differing distribution of PNNs among different cell subtypes in the mPFC compared to the PPC could result in differences in plasticity following PNN degradation in the two brain regions. In theory, this difference could have influenced the effect that PNN degradation in the mPFC versus the PPC had on TUNL performance.

3.5 The Relation of the Rodent TUNL task to Human Working Memory

TUNL has many advantages over other tasks that measure spatial WM in rats, however the validity of TUNL as a model of human spatial WM can be called into question. In human and primate studies, a heavy emphasis has been placed on visual and spatial tasks when assessing WM (Brigadoi et al. 2017; Courtney et al. 1998; Hecker and Mapperson 1997). This is logical, because humans and primates rely heavily on visual information to navigate their environment. For a long time, it has been questioned whether primates and rodents have similar evolutionary neural backgrounds with regards to WM (Tsutsui et al. 2016). Rats and humans navigate and interact with their environments in different ways, and it is possible that rats rely more on olfactory and tactile WM than visual WM. As such, direct comparisons between rat and human spatial WM may not be the most translational approach. Additionally, the ecological validity of the TUNL task can be called into question. Rats in TUNL are placed in a small box in which there are few novel olfactory or tactile cues between sessions. In the wild, however, the visual WM of rats would be combined with cues from other sensory modalities. Therefore, perhaps a multisensory WM task is the most appropriate way to measure WM in rats. The human CANTAB test for spatial WM that TUNL is based on presents similar problems to TUNL. It is not necessarily appropriate to study human spatial WM in isolation, because many socioaffective

factors such as stress can affect WM in real-life scenarios (Luethi et al. 2008; Curci et al. 2013).

With all of this being considered, TUNL still has many translational advantages in the study of WM. It is an automated test that has high face validity in the sense that the TUNL task very much resembles the CANTAB spatial WM test in humans (Bussey et al. 2012). Additionally, when compared to other non-matching tasks such as the DNMP task, it has large advantages. The TUNL task causes relatively low levels of stress to rats prior to testing because it requires little handling of animals due to its automation (Bussey et al. 2012). Additionally, its automated capabilities allow it to record large amounts of information such as response latencies, blank touches, and information regarding stimulus separations in an efficient manner (Talpos et al. 2012). To conclude, a visual WM paradigm may not be the most effective way of assessing WM in rodents, and there is value in developing a multisensory measure of WM. With that being said, TUNL has high face validity and currently appears to be the best translational task for testing spatial WM in rodents.

3.6 Future Directions

The lack of reductions in WFA intensity (Fig. 13D) and PPC PNN counts (Fig. 14E) between PEN and ChABC-treated rats makes the behavioural results following PPC manipulation difficult to interpret. In future studies, it would be valuable to assess WFA intensity in the PPC within 7 days of surgery, as was done for the mPFC (Fig. 15). This would give an idea of whether initial infusions of ChABC were successful in the PPC, and could reveal the rate at which PNNs regrow in the PPC over time. In future studies, it would also be interesting to pair ChABC infusions with MMP inhibitors in the mPFC to see if the ChABC-driven improvement in the 20 s TUNL condition can be reduced or inhibited altogether. Additionally, it would be valuable to assess how synaptic plasticity was affected following mPFC and PPC ChABC infusions. In a previous study from our lab, we used a gephyrin⁺ puncta stain and did not detect any significant changes in presynaptic terminals on inhibitory synapses following mPFC ChABC infusions (Paylor et al. 2018). In future studies, we could also use a gephyrin⁺ puncta stain in the PPC following ChABC infusions to determine whether synaptic plasticity was affected. Additionally, using whole-cell recordings to assess cell excitability following ChABC infusions into the mPFC and the PPC could be valuable in assessing the effect of ChABC on local excitatory-inhibitory balance. In future studies, it would also be valuable to study the effect that

ChABC infusions into the hippocampus have on TUNL, because like the mPFC and the PPC, the hippocampus contains PNNs and plays an important role in spatial WM.

A limitation of TUNL in the current study is that it assesses both attention and spatial WM, which are two processes that are not easily dissociated (Awh and Jonides 2001; Awh et al. 2006). In a future experiment, rats could be tested on the automated 5-Choice Serial Reaction Time Task following mPFC ChABC infusions, because it is a task that tests attention without any involvement of spatial WM (Turner et al. 2015). Based on the results of such a test, it could be concluded whether spatial WM or attention mediated the improvement in the 20 s delay TUNL task. Additionally, it would also be interesting to study the effect of mPFC ChABC infusions on a rule-switching task that measures behavioural flexibility. In the current study, rats were trained extensively in the TUNL task prior to ChABC infusions. In future studies, infusions of ChABC into the mPFC and the PPC can be done prior to rats being introduced to TUNL. PNNs play important roles in learning, and it would be interesting to test whether PNN degradation affects the rate at which rats can acquire TUNL.

ChABC infusions are an effective tool for degrading PNNs and studying the behavioural outcomes associated with such degradation. Unfortunately, however, surgical infusions of ChABC are labor intensive and there are issues with surgeries being done repeatedly throughout a rat's lifespan. Recently, an inducible viral vector has been developed in mice that can efficiently and reversibly degrade PNNs by regulated delivery of ChABC (Burnside et al. 2018). In future studies, this technology could be used to carefully control PNN density throughout postnatal development, and can be paired with TUNL to study the relationship between PNN density and spatial WM over a rodent's lifetime. With that being said, brevicin knockout mice that are deficient in PNNs do not show any changes in learning and memory (Brakebusch et al. 2002). Additionally, the use of this inducible vector lacks brain region specificity with regards to PNN degradation, and therefore depending on the research question, may not be the most effective tool in follow-up studies.

In conclusion, the results gathered from the current study suggest that PNNs in the mPFC play a subtle role in adapting to a novel delay in a spatial WM task. Additionally, it appears that PNNs in the PPC are not involved in TUNL, however this claim cannot be made with complete confidence because PNN degradation in the PPC was not confirmed 30 days post surgery. Future

experiments should help to confirm the findings of the present study, as well as test the role that PNNs in other brain regions have on learning and memory.

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